

Published in the United States of America

2017 • VOLUME 11 • NUMBER 1

AMPHIBIAN & REPTILE CONSERVATION

Angola and Africa

amphibian-reptile-conservation.org

ISSN: 1083-446X

eISSN: 1525-9153



Amphibians of Haut-Ogooué Province, southeastern Gabon

^{1,6,*}Gregory F. M. Jongsma, ²Elie Tobi, ³Graham P. Dixon-MacCallum, ⁴Abraham Bamba-Kaya, ⁴Jean-Aimé Yoga, ⁴Jean-Daniel Mbega, ⁴Jean-Hervé Mve Beh, ⁵Andrea M. Emrich, and ⁶David C. Blackburn

¹New Brunswick Museum, 277 Douglas Avenue, Saint John, NB, CANADA E2K 1E5 ²Center for Conservation and Sustainability, Smithsonian Conservation Biology Institute, CNG, Gabon Biodiversity Program ³1756 Cambridge St. Halifax, NS, CANADA, B3H 4A9 ⁴Institut de Recherches Agronomiques et Forestières, Libreville, GABON ⁵141 Wentworth Ave. Saint John, NB, CANADA, E2L 2S7 ⁶Florida Museum of Natural History, University of Florida Gainesville, Florida 32611, USA

Abstract.—We provide the most complete inventory to date of amphibians for Haut-Ogooué province in southeastern Gabon. This inventory is based on an 11-day survey conducted in 2015 around two villages, Doumaye and Mboua, near the Gabon-Congo border and a previous survey in Batéké Plateau National Park during 2011. We report 42 species of anuran amphibians (21 genera; 11 families) for Haut-Ogooué including 26 new species records for the province and two new country records for Gabon (*Afrixalus osorioi* and *Hyperolius balfouri*). This work brings the total known amphibian diversity in Gabon to 98 species.

Résumé.—Nous fournissons dans cet article, l'inventaire le plus complet des amphibiens de la province du Haut-Ogooué dans le sud-est du Gabon. Cet inventaire se fonde sur des recherches menées durant 11 jours en 2015 autour de deux villages, Doumaye et Mboua, près de la frontière Gabon-Congo et celles menées en 2011 dans le parc national des Plateaux Batéké par Zimkus & Larson (2013). Nous rapportons 42 espèces d'amphibiens (21 genres, 11 familles) pour le Haut-Ogooué dont 26 nouvelles espèces pour la province et trois nouvelles mentions pour le Gabon (*Afrixalus osorioi* et *Hyperolius balfouri*). Ce travail porte à 98 espèces le nombre total connu de la diversité des amphibiens du Gabon.

Keywords. Africa, anuran, diversity, frogs, herpetofauna, savanna, forest

Citation: Jongsma GFM, Tobi E, Dixon-MacCallum GP, Bamba-Kaya A, Yoga J-A, Mbega J-D, Mve Beh J, Emrich AM, Blackburn DC. 2017. Amphibians of Haut-Ogooué Province, southeastern Gabon. *Amphibian & Reptile Conservation* 11(1) [Special Section]: 1–23 (e144).

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Received: 06 December 2016; **Accepted:** 13 September 2017; **Published:** 20 November 2017

Introduction

Despite its relative small size, Gabon hosts a number of significant biogeographic features, including the Batéké Plateau, the Ogooué River, and two hypothesized Pleistocene forest refugia (Crystal Mountains and Chaillu Massif). The country is dominated by lowland rainforest, interspersed with a forest-savanna mosaic. Despite topographic diversity and expansive pristine habitat, there is a paucity of research that directly explores the influence and interactions of these features on biodiversity. In part, this is a result of incomplete sampling across most of Gabon. Our report adds to the growing inventory of Gabonese amphibians based on surveys of a region that is underrepresented in natural history collections.

The earliest known amphibian and reptile specimens from Gabon were collected by Charles Eugène Aubry-Lecomte. A French civil servant and keen naturalist,

Aubry-Lecomte made an important collection along the Gabonese coast for the Muséum national d'Histoire naturelle in Paris (Duméril 1856; Beolens et al. 2011). Between 1850 and 1854, he collected the first series of *Cycloderma aubryi* (Duméril 1856) and *Leptopelis aubryi* (Duméril 1856) as well as several new species of birds and plants (Duméril 1856; Beolens et al. 2011). Around the same period (1851–1855), Henry Alexander Ford, an American M.D, was stationed at Baraka Mission in present day Libreville to research malarial fever (Ford 1856). During this time, he collected reptiles for the Academy of Natural Sciences of Philadelphia (now, of Drexel University), including the type series of *Poromera fordii* (Hallowell 1857) and *Gerrhosaurus nigrolineatus* (Hallowell 1857). In 1855, Paul Belloni Du Chaillu became the first westerner to explore the interior of Gabon (Du Chaillu 1861). Funded by the Academy of Natural Sciences of Philadelphia, Du Chaillu collected large series

Correspondence. *gregor.jongsma@gmail.com

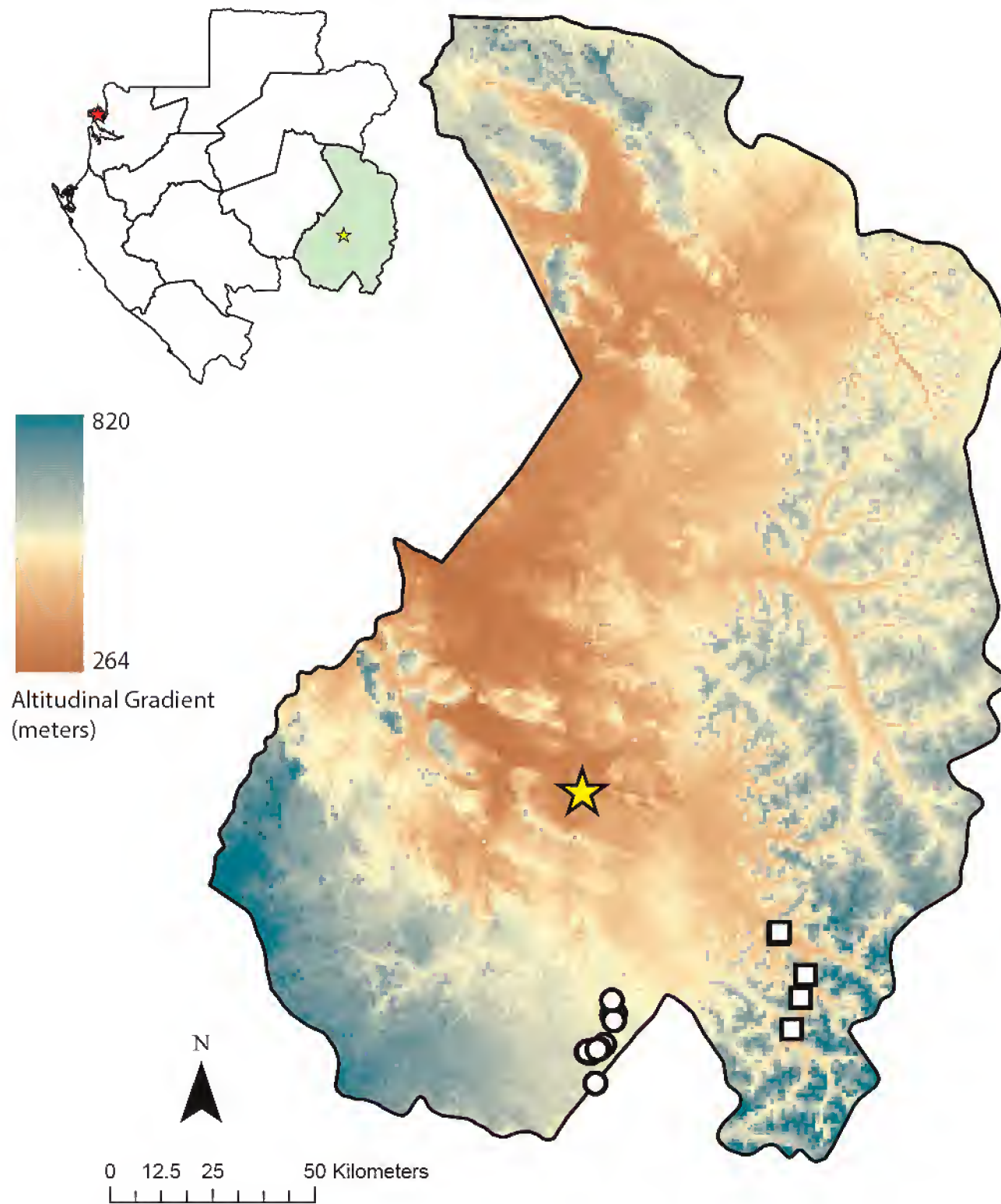


Fig. 1. Map of Haut-Ogooué province, with the capital Franceville (yellow star). Sample sites from this study are indicated with white circles. White squares denote study site for Zimkus and Larson (2013).

of birds and mammals (including the first intact gorilla specimens) and the type series of *Amnirana albolabris* (Hallowell 1856). At the turn of the 20th Century, Ernest Haug, a missionary for the Société des missions évangéliques de Paris, conducted two methodical herpetological inventories approximately 50 km southwest of Lambaréné, Moyen-Ogooué for the Muséum national d'histoire Naturelle de Paris. This resulted in 29 reptile species and 23 frog species (Mocquard 1897, 1902). Herpetological work since the early 1900s has been sporadic but there was an upsurge of inventory work around the beginning of the 21st Century (Burger et al. 2004; Burger et al. 2006; Frétey and Blanc 2000; Frétey and Dewynter 1998; Knoepffler 1966, 1974; Lötters et al. 2000; Lötters et al. 2001; Pauwels et al. 2004; Pauwels and Rödel 2007; Zimkus and Larson 2013), and Gabon's known amphibian diversity has increased substantially through these recent efforts.

At the turn of the millennium, the country amphibian total for Gabon was 72 species (Frétey and Blanc 2000). Today, less than two decades later, there are now 96 known amphibian species in Gabon, including 94

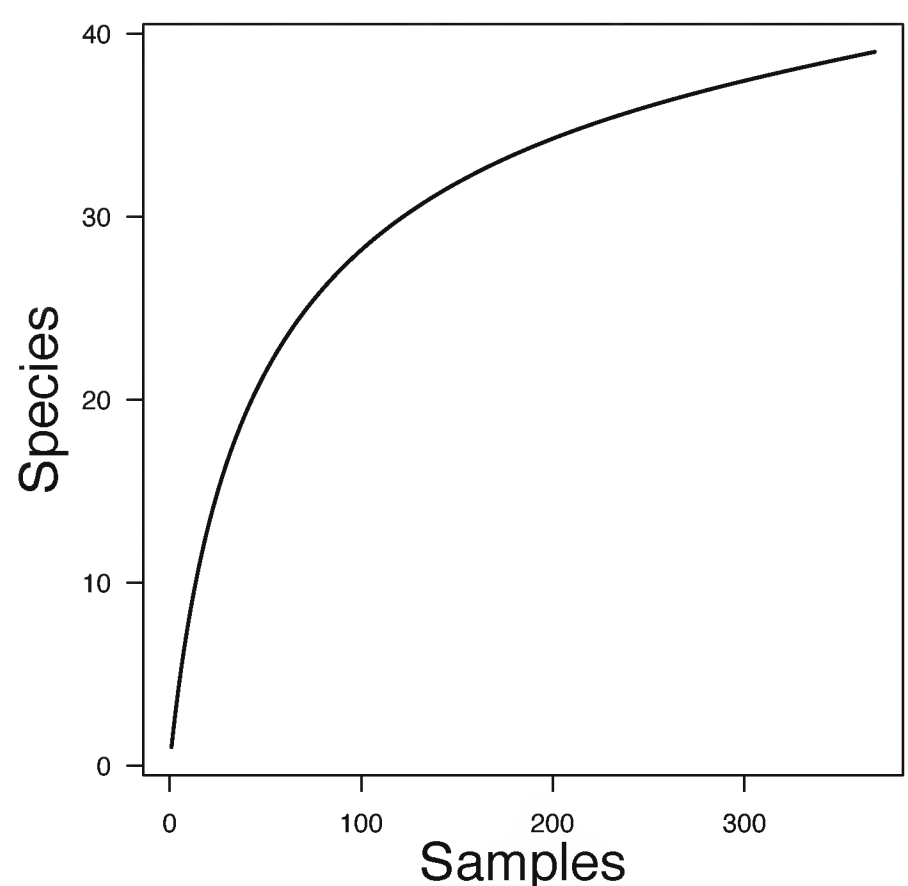


Fig. 2. Species rarefaction curve based on amphibians encountered in Haut-Ogooué province between April 21st to May 1st, 2015.



Fig. 3. Different habitats sampled in Haut-Ogooué during 2015 field surveys. Lentic habitat in closed forest (A), lotic habitat with closed forest (B, C), lentic habitat at edge of forest (D), lentic habitat in savanna (E), lentic habitat in disturbed open area (F), savanna (G), lentic habitat open forest habitat (H).

frogs and two caecilians (Neil and Jongsma 2016; Evans et al. 2015; Zimkus and Larson 2013; Frétey et al. 2011; Bell et al. 2011; Burger et al. 2006; Pauwels and Rödel 2007; Pauwels 2016). All published amphibian inventories for Gabon are restricted to just six of the 13 national parks (see summary Pauwels and Rödel 2007; Pauwels 2016). However, several recently discovered species were described from outside parks, including *Werneria iboundji* Rödel et al. 2004 and *Leptodactylodon stevarti* Rödel and Pauwels 2003. Zimkus and Larson (2013) car-

ried out the first survey of amphibians in Haut-Ogooué Province in Batéké National Park (BNP) and reported 18 frog species (three unidentified), including four new country records. Our recent survey, presented below, reveals many additional species for the province and two additional species records for Gabon. We hope that our study will serve as a guide to students and researchers undertaking future herpetofaunal work in both Gabon and Haut-Ogooué Province.

Materials and Methods

Gabon is a small equatorial country (267,667 km²) dominated by tropical moist forest (80% total land cover; Lee et al. 2006). The dominant hydrological feature is the Ogooué River basin. There are four seasons: a long rainy season from January to May; a long cold dry season from June to September; a short rainy season from October to December; and a short dry season from December to January. The average annual temperature is 26 °C (Lee et al. 2006).

Haut-Ogooué Province (36,547 km²) is located in southeastern Gabon and is composed of three major geological formations: the Chaillu Massif in the southwest, the Franceville Basin in the northwest, and the Batéké Plateau to the east. The Chaillu Massif and Batéké Plateau are unique in Gabon because of their geological histories and contemporary environments. The Chaillu Massif is an ancient formation dating to >2 billion years ago that hosts some of the highest elevation forests in Gabon, including forest refugia (Sosef 1994; Vandeweghe 2009). The massif is dominated by forest but also hosts small forest-savanna mosaics around the foothills that originate in the Haut-Ogooué Province. The Batéké Plateau has a sandy substrate and is dominated by large swaths of savanna that are contiguous with plains in southern Africa (Vandeweghe 2009). The border of Haut-Ogooué represents the boundary between three major watersheds: the Kouilou-Niari River, the Congo River, and the Ogooué River. We conducted surveys at sites within the Ogooué Basin at the foothills of the Chaillu Massif. Zimkus and Larson (2013) work was based at the Batéké Plateau, also within the Ogooué Basin.

We conducted visual encounter surveys around two villages: Doumaye (02.2402°S, 013.5812°E) on the left side of the Ogooué River, and Mboua (02.1532°S, 013.6398°E) to the right side of the river. Both sites are located in the administrative department of Lekoko in Haut-Ogooué province. The village of Doumaye is dominated by savanna habitat with gallery forest associated with rivers. The habitat around the village of Mboua consists of continuous gallery forest. We spent five survey nights in Doumaye (21–25 April 2015) and six in Mboua (26 April–1 May 2015). We typically worked between 19h00 to 00h00 each night, targeting forested streams and rivers, and small still bodies of water (Fig. 3). Our research in Haut-Ogooué Province focused on six species (*Afraxalus dorsalis*, *Amnirana albolabris*, *Hyperolius olivaceus*, *H. ocellatus*, *Phrynobatrachus africanus*, and *Scotobleps gabonicus*) for a comparative phylogeographic study around the Ogooué River. We captured other amphibians opportunistically.

All species encountered across both sites were photographed alive and swabbed for chytrid fungus (*Batrachochytrium dendrobatidis*; Bd). Voucher specimens were euthanized using an aqueous solution of MS-222,

and a sample of liver tissue was removed and stored in RNAlater, before preserving the whole specimen in 10% neutral-buffered formalin. Specimens are deposited at the California Academy of Sciences (CAS) in San Francisco, California, Sam Noble Museum (OMNH) in Norman, Oklahoma, and Gabon's national collection in Yenzi Camp, Gamba, Gabon. We refer to specimens in Gabon's collection using GFMJ field numbers. To determine the extent to which our species sampling was comprehensive, we constructed a rarefaction curve using the rare curve function in the vegan package (Oskansen et al. 2013) for R (R Core Team 2013).

Laboratory work was conducted at the Florida Museum of Natural History (FLMNH) by GFMJ. We extracted genomic DNA from tissues (liver, muscle, or toe clips) using Qiagen DNeasy Kits following their protocol for animals. Using polymerase chain reaction (PCR), we amplified a ~762 base pair (bp) fragment of mitochondrial DNA that encodes part of the mitochondrial ribosomal 16S gene (94 °C 30 s, 52 °C 30 s, 72 °C one min) using 35 cycles and the oligonucleotide primers 16Sc and 16Sd (Moriarty and Cannatella 2004). We used ExoSAP-IT (Affymetrix) to purify all amplified PCR products and then shipped this product for Sanger sequencing at Genewiz Co. All sequences are deposited in GenBank (accession numbers: MF537671–MF537697).

Species Accounts

AMPHIBIA – Frogs

ARTHROLEPTIDAE

Arthroleptis cf. poecilonotus (Peters 1863)

Material: One (1) specimen. Doumaye: CAS 258166. Fig. 4A.

Comments: *Arthroleptis poecilonotus* is a leaf-litter species that is associated with forest habitats but also found in wet savanna and near human habitations. It is widespread across West and Central Africa and is likely composed of several unnamed species (Blackburn 2008). Populations in Central Africa, including eastern Nigeria, Cameroon, Gabon, and Republic of Congo, referred to *A. poecilonotus* are not conspecific with those identified as the same species in western Africa (Blackburn et al. 2010), though no taxonomic changes have yet been made. This species was first reported for Gabon by Mocquard (1902; under *Arthroleptis inguinalis*) near Lambaréné in Moyen-Ogooué province. It has since been found in several national parks including: Batéké NP (Zimkus and Larson 2013), Crystal Mountains NP (Löters et al. 2001), Ivindo National Park (Frétey and Blanc 2000), Loango NP (Burger et al. 2006), and Lopé NP (Frétey and Blanc 2001).

Amphibians of southeastern Gabon

Table 1. Amphibian species recorded for Haute-Ogooué Province. *=New provincial record. **=New country record. Habitats include forest (F), open disturbed areas (O), savanna (S), and edge (ED). Microhabitats include leaf litter (LL), arboreal (AR), and aquatic (AQ). Some species lacking microhabitat information were not collected by the authors.

	Doumaye	Mboua	Zimkus and Larson 2013	Habitat	Microhabitat
ARTHROLEPTIDAE					
<i>Arthroleptis</i> cf. <i>poecilonotus</i>	X		X	F	LL
<i>A.</i> cf. <i>sylvaticus</i>		X	X	F	LL
<i>Astylosternus batesi</i> *	X	X		F	LL
<i>Cardioglossa gracilis</i> *	X	X		F	LL
<i>Leptopelis aubryi</i> *	X			O	AR
<i>L. aubryioides</i> *		X		F	AR
<i>L. calcaratus</i> *	X	X		F	AR
<i>L. millsoni</i> *	X	X		F	AR
<i>L. notatus</i> *		X		F	AR
<i>L. ocellatus</i> *	X	X		F	AR
<i>Scotobleps gabonicus</i> *	X	X		F	LL
BUFONIDAE					
<i>Sclerophrys gracilipes</i> *	X	X		F	LL
<i>S. superciliaris</i> *	X			F	LL
CONRAUIDAE					
<i>Conraua crassipes</i> *		X		F	AQ
DICROGLOSSIDAE					
<i>Hoplobatrachus occipitalis</i>	X		X	S	AQ
HYPEROLIIDAE					
<i>Afrixalus dorsalis</i> *	X	X		O	AR
<i>A. osorioi</i> **	X			O	AR
<i>A. quadrivittatus</i>	X		X	O	AR
<i>Cryptothylax greshoffi</i>		X	X	O	AR
<i>Hyperolius adspersus</i>	X		X	S	AR
<i>H. balfouri</i> **	X	X		O	AR
<i>H. bolifambae</i>			X	F	
<i>H. kuligae</i> *		X		F	AR
<i>H. ocellatus</i> *	X	X		F/ED	AR
<i>H. olivaceus</i> *	X			S	AR
<i>H. pardalis</i> *	X	X		S/ED	AR
<i>H. phantasticus</i> *	X			S	AR
<i>Kassina maculosa</i>			X	S	AR
<i>Opisthothylax immaculatus</i> *		X		F	AR
<i>Phlyctimantis leonardi</i> *	X			S	AR
PHRYNOBATRACHIDAE					
<i>Phrynobatrachus africanus</i> *	X	X	X	F	LL
<i>P. horsti</i> (<i>P. ruthbeateae</i>)			X	F	LL
PIPIDAE					
<i>Hymenochirus boettgeri</i>			X	F	AQ
<i>Xenopus pygmaeus</i>			X	F	AQ
PTYCHADENIDAE					
<i>Ptychadena perreti</i>			X	S	
<i>P. taenioscelis</i>			X	S	
<i>P. uzungwensis</i>			X	S	

Table 1 (continued). Amphibian species recorded for Haute-Ogooué Province. *=New provincial record. **=New country record. Habitats include forest (F), open disturbed areas (O), savanna (S), and edge (ED). Microhabitats include leaf litter (LL), arboreal (AR), and aquatic (AQ). Some species lacking microhabitat information were not collected by the authors.

	Doumaye	Mboua	Zimkus and Larson 2013	Habitat	Microhabitat
PYXICEPHALIDAE					
<i>Aubria masako</i> *		X		F	AQ
RANIDAE					
<i>Amnirana albolabris</i>	X	X	X	F	AR
<i>A. amnicola</i> *	X	X		F	AR
<i>A. lepus</i> *	X	X		F	AR
RHACOPHORIDAE					
<i>Chiromantis rufescens</i>		X		F	AR

***Arthroleptis cf. sylvaticus* (Laurent 1954)**

Material: Six (6) specimens. Doumaye: CAS 258184; GFMJ 1327. Mboua: CAS 258166, 258241–42; OMNH 44767. Fig. 4B.

Comments: *Arthroleptis sylvaticus* is a leaf-litter species that is widespread across Central Africa, north of the Congo River and is a complex of several undescribed species. This species is known from the following national parks: Batéké (Zimkus and Larson 2013), Ivindo (Frétey and Blanc 2000), Loango (Burger et al. 2006), Lopé (Frétey and Blanc 2001), and Moukalaba-Doudou (Burger et al. 2004).

***Astylosternus batesi* (Boulenger 1900)**

Material: Eighteen (18) specimens. Doumaye: CAS 258139, 258151, 258211–12; GFMJ 1240, 1242, 1270, GFMJ 1322–23, 1372. Mboua: CAS 258285–86; OMNH 44768–69; GFMJ 1397, 1461, 1514–15. Fig. 4C, D.

Comments: *Astylosternus batesi* is a leaf-litter species that is strongly associated with forested streams. Individuals are best detected by eye-shine at night. This species is distinguishable from the closely related and sympatric species *Scotobleps gabonicus* by its smoother skin. It is known from three national parks: Crystal Mountains NP (Lötters et al. 2001), Ivindo NP (Frétey and Blanc 2000), and Moukalaba-Doudou NP (Burger et al. 2004).

***Cardioglossa gracilis* (Boulenger 1900)**

Material: Ten (10) specimens. Doumaye: CAS 258182–83, 258197, 258208, 258210; GFMJ 1326. Mboua: CAS 258227, 258251; OMNH 44770–71. Fig. 4E

Comments: Male *C. gracilis* are typically found calling from leaf litter within 10 meters of forested streams with sloped sides. Their call is an insect-like click. Males are faithful to their calling sites (GFMJ, pers. obs.); if disturbed, they will vacate the calling site, but

then return to the same spot a short while later. This species was first collected in neighboring Equatorial Guinea by the ornithologist George L. Bates along the Benito River (Boulenger 1900). *Cardioglossa gracilis* is also known from Ivindo NP (Frétey and Blanc 2000) and Moukalaba-Doudou NP (Burger et al. 2004). We also collected voucher specimens from Mitoné village near Lambaréné (000.64375°S, 010.22071°E; CAS 258016) and Madoukou village near Lastoursville (00.86831°S, 12.67244°E; GFMJ 1583).

***Leptopelis aubryi* (Duméril 1856)**

Material: Six (6) specimens. Doumaye: CAS 258202, 258260–61; GFMJ 1470–71, 1473. Fig. 4F.

Comments: *Leptopelis aubryi* was originally collected by Charles Eugène Aubry-Lecomte in the early 1850s and is among the first amphibians ever collected in Gabon (Duméril 1856). We encountered all individuals in tall grass in ditches and around well pumps in Doumaye. It is a disturbance specialist. In the Plaine Ouanga Reserve in the Gamba Complex of Protected Areas (GCPA) in the Ogooué-Maritime province, one of us (E. Tobi) regularly finds this species on the branches of trees at the edge of forest and in the forest. It is widespread across Central Africa, North of the Congo River. Within Gabon voucher specimens are known from the following national parks: Crystal Mountains NP (Lötters et al. 2001), Ivindo NP (Frétey and Blanc 2000), Loango NP (Burger et al. 2006), Lopé NP (Frétey and Blanc 2001), and Moukalaba-Doudou (Burger et al. 2004).

***Leptopelis aubryioides* (Andersson 1907)**

Material: One (1) specimen. Mboua: CAS 258234. Fig. 4G, H.

Comments: A single individual was encountered near a small forest stream next to a foot path. This species is easily distinguished from similar species by the distinct spurs on its heels and its small size (Amiet 2012). The

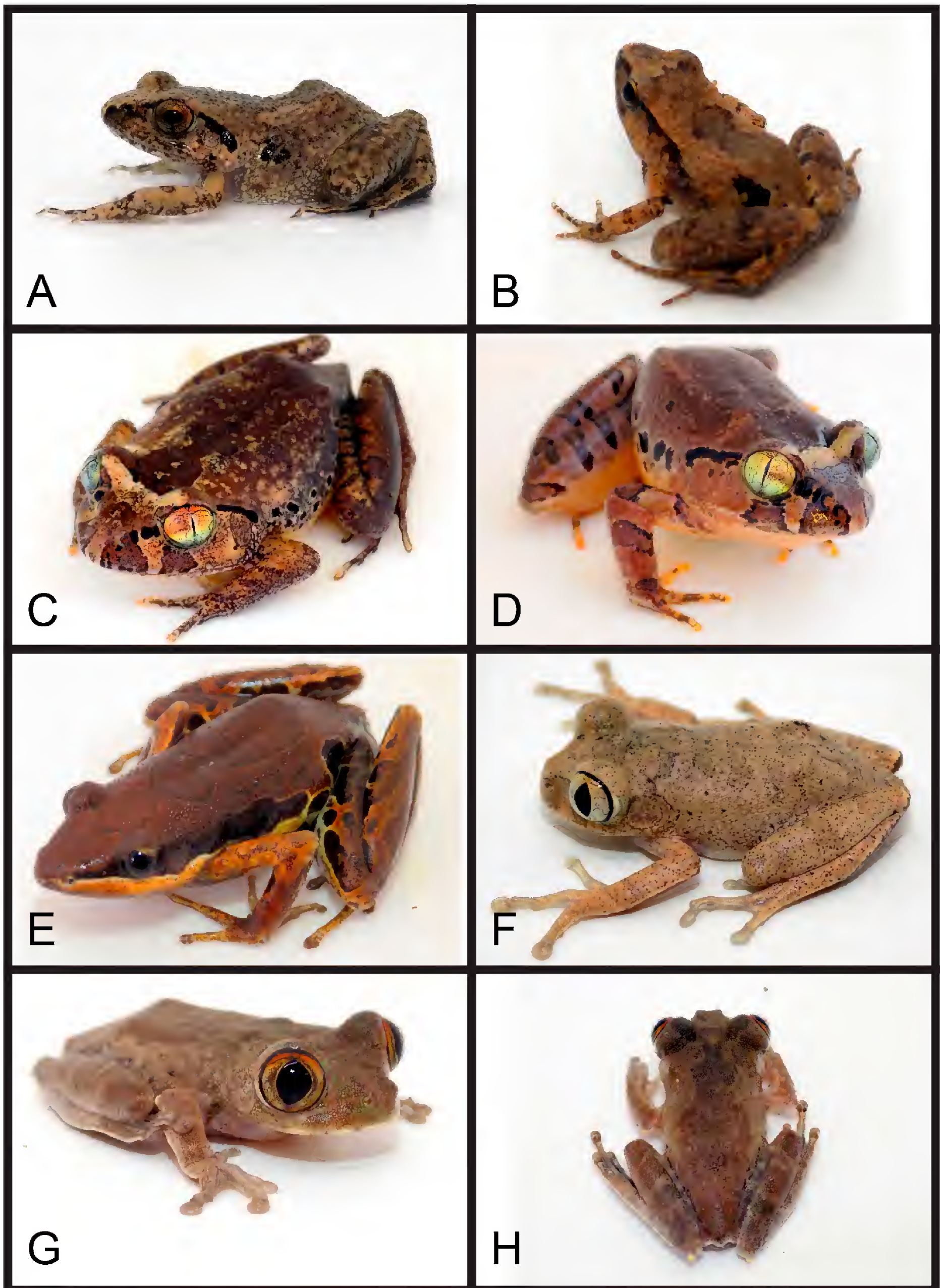


Fig. 4. *Arthroleptis* cf. *poecilonotus* CAS 258166 (A), *A. sylvaticus* CAS 258184 (B), *Astylosternus batesi* CAS 258139, GFMJ 1240 (C, D), *Cardioglossa gracilis* CAS 258016 (E), *Leptopelis aubryi* 258261 (F), *L. aubryioides* CAS 258234 (G, H).

similar sympatric species, *L. aubryi*, lacks heel spurs and is found in more disturbed habitats. This species is known across Cameroon (Amiet 2012) but few records exist for Gabon. Within Gabon, it is known from: Ivindo NP (Bell et al. 2011), Loango NP (Burger et al. 2006, listed as *L. omissus*), and Lopé NP (Frétey and Blanc 2001 listed as *L. omissus*). We sequenced 16S rRNA of CAS 258234, and confirmed its identification using BLAST (GenBank accession: MF537690; nearest GenBank sequence is KT967084.1; 96% identical).

***Leptopelis calcaratus* (Boulenger 1906)**

Material: Seven (7) specimens. Doumaye: CAS 258148, 258190–91, 258274, GFMJ 1340. Mboua: CAS 258253–54. Fig. 5A.

Comments: *Leptopelis calcaratus* is an arboreal species that is widespread across Central Africa (Cameroon, Gabon, Republic of Congo, and DRC). Within Gabon, it is known from Crystal Mountains NP (Lötters et al. 2001), Ivindo NP (Frétey and Blanc 2000), and Moukalaba-Doudou (Burger et al., 2004). We encountered six of the seven individuals perched in trees above four meters from the ground adjacent to forested streams. It is easily identified by the spur on its heel and larger size than *L. aubryioides*.

***Leptopelis millsoni* (Boulenger 1895)**

Material: Three (3) specimens. CAS 258147, OMNH 44774. Mboua: CAS 258233. Fig. 5B, C.

Comments: We found individuals 1–2.5 m above the ground along forested streams. This species is closely associated with streams. This arboreal species is widespread across Central Africa (Cameroon, Gabon, Republic of Congo, and DRC). Within Gabon, it is known from Crystal Mountains NP (Lötters et al. 2001; Bell et al. 2011), Ivindo NP (Frétey and Blanc 2000; Bell et al. 2011), and Moukalaba-Doudou (Burger et al. 2004). In addition to this new Haut-Ogooué record, we also found this species in Moyen-Ogooué (CAS 257990–91, 258049, 258076, 258119, 258126) and Ogooué-Lolo (CAS 258303) provinces.

***Leptopelis notatus* (Peters 1875)**

Materials: Five (5) specimens. Mboua: CAS 258230–32, 258283; GFMJ 1405. Fig. 5D–F.

Comments: This arboreal species is widespread across Central Africa and we found it near streams 1–2 m above the ground. We encountered three females and two males. The females were all uniformly green, and the males were mottled green and light brown. *Leptopelis notatus* is known from the following national parks: Crystal

Mountains NP (Lötters et al. 2001), Ivindo NP (Frétey and Blanc 2000), and Moukalaba-Doudou (Burger et al. 2004).

***Leptopelis ocellatus* (Mocquard 1902)**

Material: Nine (9) specimens. Doumaye: CAS 258189, 258196; GFMJ 1337. Mboua: CAS 258252; GFMJ 1422, 1431, 1456–57; OMNH 13751. Fig. 5G.

Comments: *Leptopelis ocellatus* is associated with slow rivers and forested swamps. They were found between 10 cm and one m above the ground or water. Across Gabon, this species is known from Ivindo NP (Frétey and Blanc 2000) and Moukalaba-Doudou NP (Burger et al. 2004). We also encountered this species around Junkville, Moyen-Ogooué (CAS 258134–35) and around Ogooué-cinq and Madoukou villages near Lastoursville, Ogooué-Lolo (CAS 258287, 258306, 258316).

***Scotobleps gabonicus* (Boulenger 1900)**

Material: Twelve (12) specimens. Doumaye: CAS 258149, 258150; GFMJ 1239, 1267–68, 1350, 1367. Mboua: CAS 258228–29; GFMJ 1399, 1401–02. Fig. 5H.

Comments: *Scotobleps gabonicus* is found in leaf litter, close to stream edges with sandy to pebbly substrates. While found near streams, we never observed individuals ($n = 84$ across Gabon) to leap into the water when approached; when detected, it either remains in place or moves in a direction other than the stream. This species appears to prefer clear streams as we did not find it near sections with muddy water. This may suggest that its reproduction and life history are dependent on specific stream qualities, though its tadpoles remain unknown. Adults are best detected at night by eye-shine. *Scotobleps gabonicus* is widespread and common across the lower Guinean forest (Cameroon, Equatorial Guinea, and Gabon; Portik et al. 2017). In Gabon, it is known from Crystal Mountains NP (Lötters et al. 2001), Lopé NP (Frétey and Blanc 2001), Ivindo NP (collected by Bell and Stuart in 2011; NCSM 78914–15), and Moukalaba-Doudou NP (Burger et al. 2004).

BUFONIDAE

***Sclerophrys gracilipes* (Boulenger 1899)**

Material: Five (5) specimens. Doumaye: CAS 258175. Mboua: CAS 258257, 258282; OMNH 44780; GFMJ 1506. Fig. 6A.

Comments: This is a common terrestrial species in lowland forests. All individuals were encountered associated with small to medium-sized, forested streams. This

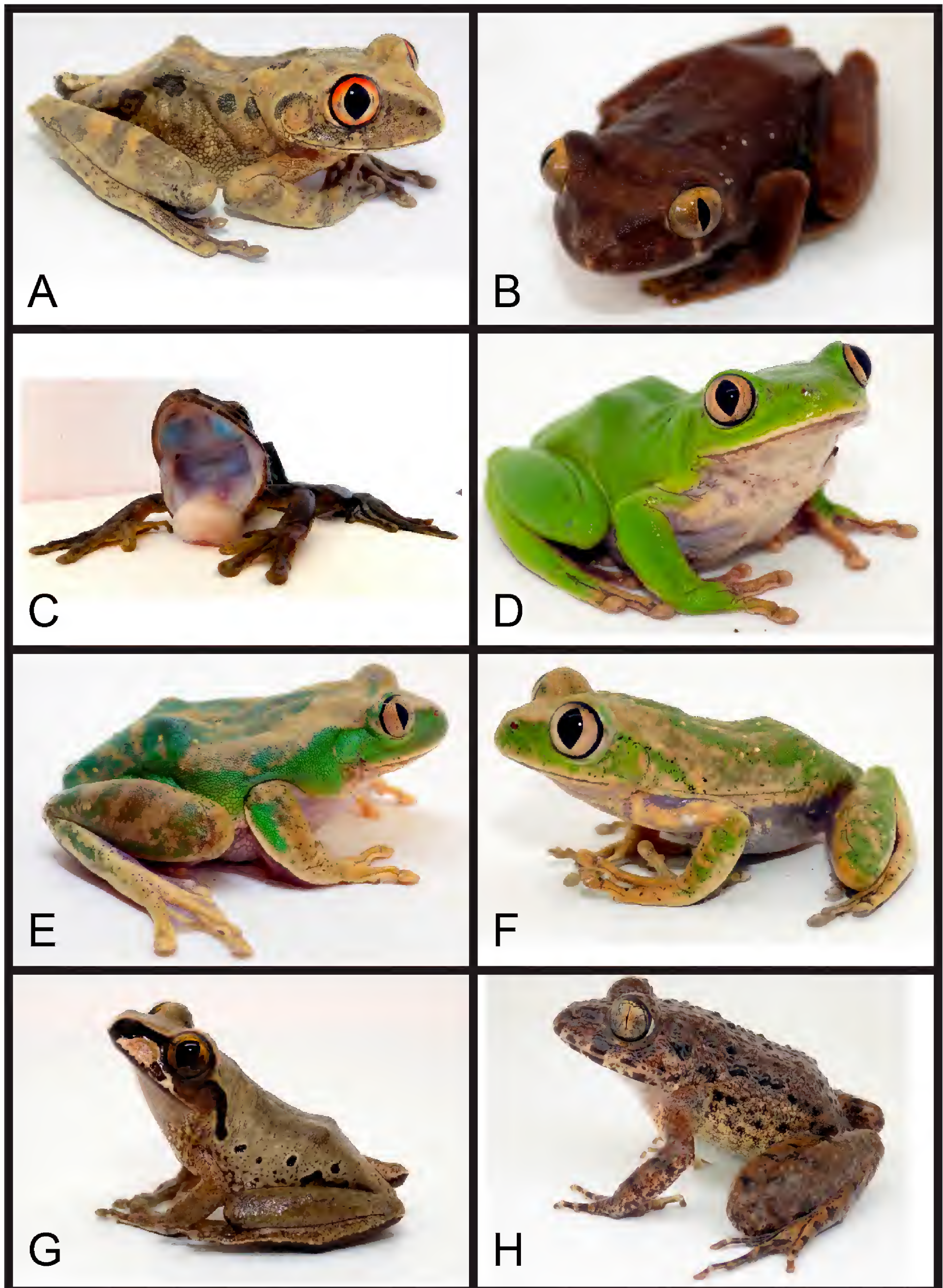


Fig. 5. *Leptopelis calcaratus* CAS 258148 (A), *L. millsoni* CAS 257991, 257990 (B, C), *L. notatus* GFMJ 1495, CAS 258283, 258230 (D–F), *L. ocellatus* GFMJ 1431 (G), *Scotobleps gabonicus* GFMJ 1350 (H).

species is widespread across Central Africa, north of the Congo River. In Gabon, this species is known from Ivindo NP (Frétey and Blanc 2000), Loango NP (Burger et al. 2006), and Moukalaba-Doudou NP (Burger et al. 2004). In 2015 we also collected vouchers in Moyen-Ogooué province near Lambaréné (CAS 257988, 257994) and Ndjolé (CAS 258051, 258096). This species was collected along Onangué Lake in 2013 (by R. Bell and B. Stuart; CAS 254506–10).

***Sclerophrys superciliaris* (Boulenger 1888)**

Material: One (1) specimen. Doumaye: GFMJ 1314. Fig. 6B, C.

Comments: One individual was brought to our camp by a local civil servant, Eric Dipanda, who found the toad on the main dirt road running through Doumaye (02.23373°S, 013.60008°E). This is the first provincial record and most southern record for the species (Barej et al. 2011), although Frétey and Blanc (2000) reports the species from the Republic of Congo without precise locality information. It is best detected by its bright eye-shine in the dark (DCB, pers. obs.).

CONRAUIDAE

***Conraua crassipes* (Buchholz and Peters 1875)**

Material: Two (2) specimens. Mboua: CAS 258277–78. Fig. 6D, E.

Comments: *Conraua crassipes* is closely associated with forested small tributaries of the Ogooué River. Individuals were found in shallow muddy substrate near the banks and detected by their eye-shine. This species is known from the following national parks: Crystal Mountains (Lötters et al. 2001), Ivindo (Frétey and Blanc 2001), Lopé (Frétey and Blanc 2000), and Moukalaba-Doudou (Burger et al. 2004). In 2015, we also encountered this species near Lambaréné (CAS 257993, 258019), Ndjolé (CAS 258084), Junkville (CAS 258122–23), and Lastoursville (CAS 258317).

DICROGLOSSIDAE

***Hoplobatrachus occipitalis* (Günther 1858)**

Material: Three (3) specimens. Doumaye: CAS 258174; OMNH 44786; GFMJ 1294. Fig. 6F.

Comments: This highly aquatic species is associated with permanent bodies of water in savanna habitat and tolerant of disturbance (Guibé and Lamotte 1958; Rödel 2000). We encountered this species at pristine savanna lakes as well as disturbed permanent pools in the village of Bafounou. This species is widely distributed across

Africa, from Senegal to Ethiopia and south to Zambia and Angola (Rödel 2000). Within Gabon, *H. occipitalis* is known from: Batéké NP (Zimkus and Larson 2013), Crystal Mountains NP (Lötters et al. 2001), and Loango NP (Burger et al. 2006). It is also known from the following areas: Ivindo, Rougier Gabon Forestry Concession (NCSM 78971–74) and from the Ouanga Plains, Basse-Banio in Nyanga province (USNM 580613–17).

HYPEROLIIDAE

***Afrixalus dorsalis* (Peters 1875)**

Material: Five (5) specimens. Doumaye: CAS 258200, 258201, 258240, 258284; GFMJ 1353. Fig. 6G.

Comments: *Afrixalus dorsalis* is a disturbance specialist, and we found individuals concentrated near village water pumps, calling from tall grasses. This species was found in sympatry with *Leptopelis aubryi*. Its identification is based on the key by Frétey et al. (2011), including the brown mediodorsal band that widens and spreads towards the eyelids (see Fig. 6G). *Afrixalus dorsalis* is known from the following national parks: Ivindo, Lopé (Frétey and Blanc 2000, 2001), Loango, Moukalaba-Doudou (Burger et al. 2004, 2006).

***Afrixalus osorioi* (Ferreira 1906)**

Material: Twenty one (21) specimens. Doumaye: CAS 258160, 258161, 258262–70; OMNH 44788; GFMJ 1356–58, 1475–78, 1531–32. Fig. 6H, Fig. 7A, B.

Comments: *Afrixalus osorioi* is similar in appearance and habitat preference to *A. dorsalis*, but distinguished from that species by its distinct advertisement call. *Afrixalus osorioi* is known from Angola, Republic of Congo, Democratic Republic of Congo, Kenya, and Uganda. These specimens represent the first country records for Gabon. This species inhabits bushland habitat. It has a unique pattern that helps distinguish it from other Gabonese *Afrixalus*, typically with a rectangular dark dorsal spot and a narrow light dorsal pattern extends to the anus (Schiotz 1999). Laurent (1982) mentions that this pattern does not vary within populations; however, we encountered some variation, including individuals that possessed no dark rectangle at all (Fig. 6, 7). The identification of these specimens was confirmed by comparing DNA sequence data for 16S ribosomal RNA from these specimens to another identified sample (K. Charles and D. Portik, unpubl. data; CAS 256140).

***Afrixalus quadrivittatus* (Werner 1908)**

Material: Three (3) specimens. Doumaye: CAS 258271–72, GFMJ 1492. Fig. 7C.

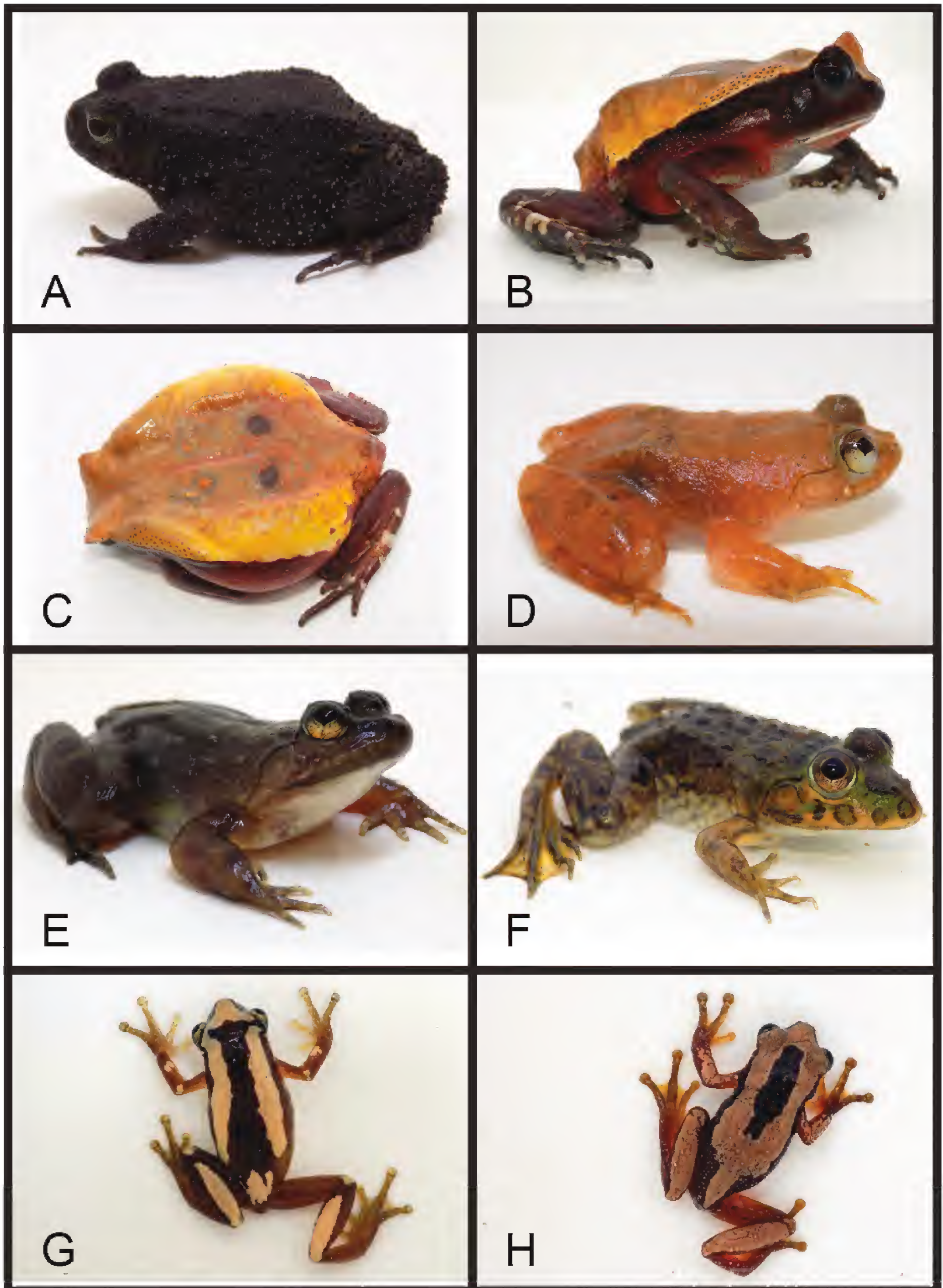


Fig. 6. *Sclerophrys gracilipes* CAS 258282 (A), *Sclerophrys superciliaris* GFMJ 1314 (B, C), *Conraua crassipes* ORB 97 (D, E), *Hoplobatrachus occipitalis* CAS 258278 (F), *Afrixalus dorsalis* ORB 140 (G) *A. osorioi* GFMJ 1356 (H).

Comments: This species is found in open grassy habitat. In Doumaye, we found individuals in a flooded area near the football stadium (02.23300°S, 13.60305°E), calling from within bunches of grasses between 10–50 cm above the ground or shallow water. At this site, *A. quadrivittatus* is sympatric with *A. osorioi*, *Hoplobatrachus occipitalis*, and *Phlyctimantis leonardi*. This species is known from Batéké NP (Zimkus and Larson 2013). We sequenced 16S rRNA of CAS 258271 and GFMJ 1492, and confirmed their identification using BLAST (GenBank accession: MF537696–97; nearest GenBank sequence is KF178889.1; 99% identical).

***Cryptothylax greshoffi* (Schilthuis 1889)**

Material: Eleven (11) specimens. Mboua: CAS 258214–216; OMNH 44789–90; GFMJ 1430, 1442–46. Fig. 7D.

Comments: We found *C. greshoffi* in open grassy habitat bordered by forest at a dammed section of the stream where locals did laundry. At night, individuals were found calling within the vegetation surrounding the water, ranging from 5 to 100 cm above the water. This species is known from Ivindo NP (Frétey and Blanc 2001) and Batéké Plateau NP (Zimkus and Larson 2013).

***Hyperolius adspersus* (Peters 1877)**

Material: Twelve (12) specimens. Doumaye: CAS 258167–69, 258332–33, 258170; GFMJ 1303–06. Fig. 7E–G.

Comments: We found *H. adspersus* in open, natural savanna habitat. Individuals called in high densities from grasses in and at the edge of shallow ponds. This species was previously considered a part of the *Hyperolius nasutus* complex (Channing et al. 2013). This species is known from Batéké Plateau NP, Loango NP (Burger et al. 2006), Moukalaba-Doudou NP (Burger et al. 2004), and Pongara NP (Pauwels 2016). It is also known from Libreville (Gratwick et al. 2011).

***Hyperolius balfouri* (Werner 1908)**

Material: Four (4) specimens. Doumaye: CAS 258171–73; GFMJ 1307. Fig. 8E.

Comments: We encountered this species at one site, a pond in savanna habitat near Doumaye (Fig. 3). It was found in open savanna calling from the edge of a pool. *Hyperolius adspersus* also occurred at this same site. This is a new country record for Gabon. It is distinguishable from other Gabonese species based on its green dorsolateral lines and the presence of a vertebral stripe (Fig. 8). The nearest known population is 715-km north in Cameroon (Amiet 2012) and represented by the subspecies *H. b. viridistriatus*. However, based on our

16S sequence data, this is more closely related to populations in East Africa, including ~2,180 km east in Mabira, Uganda (CAS 256187). We sequenced the 16S rRNA of CAS 258171–73 and GFMJ 1307 (GenBank accession: MF537676–79), and confirmed their identification using an unpublished dataset (Portik; 100% identical to CAS 256187).

***Hyperolius kuligae* (Mertens 1940)**

Material: Four (4) specimens. Doumaye: CAS 258238–39, 258247, GFMJ 1418. Fig. 8F, G.

Comments: These individuals were found within closed forest on vegetation surrounding a still section of river that was dammed for manioc fermentation. The males exhibited a bright yellow coloration at night, which helps distinguish it from similar species like *H. platyceps* (Amiet 2012). This species is known from Ivindo NP (Bell et al. 2011), Loango NP (Burger et al. 2006), and Moukalaba-Doudou (Burger et al. 2004).

***Hyperolius ocellatus* (Günther 1858)**

Material: Ten (10) specimens. Doumaye: CAS 258142, 258176–77, GFMJ 1246, 1248. Mboua: CAS 258237, GFMJ 1411, 1414–15, 1417. Fig. 8H, 9A–C.

Comments: This dichromatic species is found in the forest or at the forest-edge, typically on leaves 1–2 m above the ground and near slow sections of streams. Males are green with light dorsolateral stripes and a pale triangle on the snout that is diagnostic of the species. Females range in coloration from silvery grey to red with black spots (Schjötz 1999; Amiet 2012). *Hyperolius ocellatus* is widespread and common across Central Africa, including Gabon. To date, it is known from four national parks: Ivindo (Bell et al. 2011), Loango (Burger et al. 2006), Moukalaba-Doudou (Burger et al. 2004), and Crystal Mountains (Bell et al. 2011). It was also encountered near Mitoné (CAS 258083; -0.641950 10.217420) and Ndjolé (CAS 257997; -0.193950, 10.784770) in Moyen-Ogooué, and from Basse-Bania department in Nyanga province (USNM 558547; -3.23331, 10.619).

***Hyperolius olivaceus* (Peters 1876)**

Material: Ten (10) specimens. Doumaye: CAS 258158–59; GFMJ 1489–91. Mboua: CAS 258217–18; GFMJ 1380–82. Fig. 7H.

Comments: This disturbance specialist is common across Gabon. This species was until recently considered a subspecies of the very widespread *Hyperolius cinnamomeoventris*. However, it was recently elevated based on molecular, ecological, and phenotypic data (Bell et al. 2017). It is known from Loango (Burger et

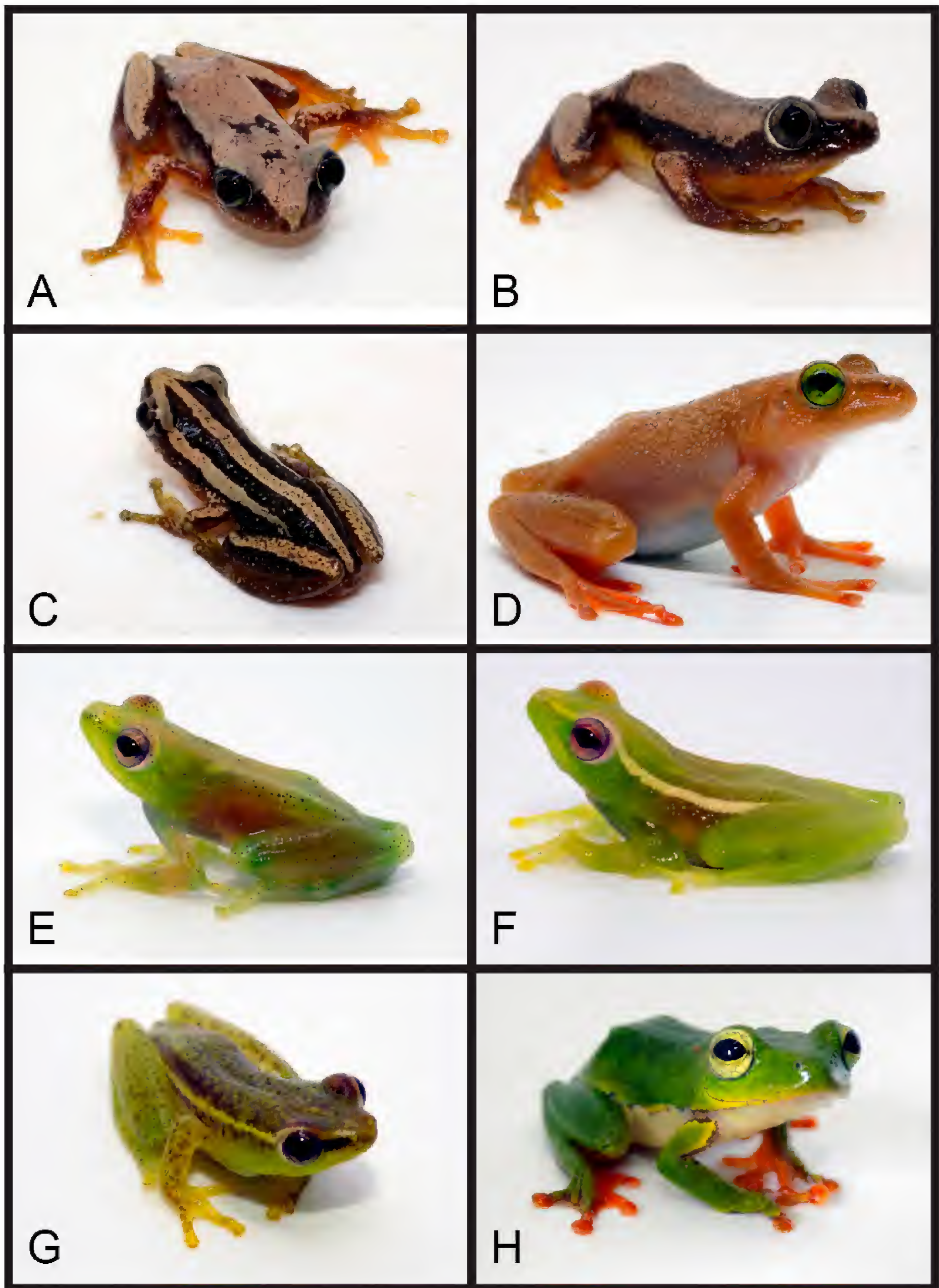


Fig. 7. *Afrixalus osorioi* CAS 258270, CAS 258161 (A, B), *A. quadrivittatus* GFMJ 1492 (C), *Cryptothylax greshoffi* OMNH 44789 (D), *Hyperolius adspersus* CAS 258168, 2583332, GMFJ 1304 (E–G), *H. olivaceus* CAS 258159 (H).

al. 2006), Moukalaba-Doudou (Burger et al., 2004), and from Libreville (Gratwick et al. 2011). We encountered individuals around artificial bodies of water in grasses and palm fronds, but never within closed canopy forests. We sequenced 16S rRNA from GFMJ 1489–91 and confirmed their identification using BLAST (GenBank accession: MF537693–95; closest GenBank sequence is MF376266; 100% identical).

***Hyperolius pardalis* (Laurent 1948)**

Material: Twenty (20) specimens. Doumaye: CAS 258162, 258203–06, OMNH 44794–99, GFMJ 1281, 1363. Mboua: CAS 258219–20, GFMJ 1447–51. GFMJ Fig. 9D–H.

Comments: We encountered *H. pardalis* in disturbed areas. One female was found on a tent, where we camped in a field in Doumaye. All other individuals were encountered in vegetation next to a small fish pond, adjacent to a forested stream. Of 17 males encountered, two males demonstrated the F-phase representing 11.8% of the male population sampled (Fig. 6 D–H). This species can be distinguished from the similar species *Hyperolius bolifambae* by its distinct vocal sac, which is pearl-white posterior to the gular gland (Amiet 2012). In Gabon, *Hyperolius pardalis* is known from Crystal Mountains NP (Löters et al. 2001), Rabi-Toucan (Burger et al. 2006), and Ivindo NP (Frétey and Blanc 2001).

***Hyperolius phantasticus* (Boulenger 1899)**

Materials: Three (3) specimens. Doumaye: CAS 258163–65. Fig. 10A–C.

Comments: *Hyperolius phantasticus* was found in small trees next to a large savanna pond, 2–2.5 meters above the ground. In the Plaine Ouanga Reserve in the Gamba Complex of Protected Areas, this species was found in shrubs close to or next to savanna ponds. This species is known from Crystal Mountains NP (Löters et al. 2001; Bell et al. 2011) and Loango NP (Burger et al. 2006). We sequenced 16S rRNA of CAS 258165 and confirmed its identification using BLAST (GenBank accession: MF537674; closest BLAST sequence is FJ594099; 97% identical).

***Opisthothylax immaculatus* (Boulenger 1903)**

Material: One (1) specimen. Mboua: CAS 258235. Fig. 10D.

Comments: This monotypic genus is distinguished from other Hyperoliidae by the combination of its vertical pupils, rough skin, and orange color (Schjötz 1999). The males have large gular glands and non-descendible

vocal sacs (Schjötz 1999). This arboreal species builds foam nests (Amiet 1991). This species was encountered at night on a stem one m above the ground, between a forested stream and an elephant wallow. In Gabon, it is known from Ivindo NP (Bell et al. 2011) and Rabi-Toucan (Burger et al. 2006). We sequenced 16S rRNA of this individual and confirmed its identification using BLAST (GenBank accession: MF537682; the most similar GenBank sequence is KX492629; 98% identical).

***Phlyctimantis leonardi* (Boulenger 1906)**

Material: Three (3) specimens. Doumaye: CAS 258209, 258273; GFMJ 1369. Fig. 10E, F.

Comments: This large hyperoliid frog was encountered in shrubs or trees, 1–2 m above the ground and near still bodies of water in open habitat. In Gabon, *P. leonardi* is common and widespread. It is known from the following national parks: Crystal Mountains (Löters et al. 2001), Ivindo, Lopé (Frétey and Blanc 2000, 2001), Loango, and Moukalaba-Doudou (Burger et al. 2004, 2006). It is also known from Basse-banio, Nyanga (USNM 580612; -3.0876, 10.4285), Junkville (CAS 258130–31; -0.051710, 11.166210), and near Ndjolé, (CAS 258063–65; -0.18482, 10.77727) in Moyen-Ogooué.

PHRYNOBATRACHIDAE

***Phrynobatrachus africanus* (Hallowell 1868)**

Material: Thirteen (13) specimens. CAS 258187–88, GFMJ 1332–34. Mboua: CAS 258222, 258243, 1425, 1427–28, 1463–65. Fig. 11 D–E.

Comments: The distantly related genera *Phrynobatrachus* and *Arthroleptis* have often proved difficult for field researchers to distinguish. The most reliable diagnostic feature is the presence of a tubercle roughly in the middle of the tarsus in *Phrynobatrachus* in addition to both an inner and outer metatarsal tubercle (Zimkus and Blackburn 2008). *Phrynobatrachus africanus* is a common forest species found in the leaf litter and easily identified by its rugose skin, yellow legs, and in males both a large flat nuptial pad and odontoid processes. We also encountered individuals with red legs in sympatry with the yellow-legged individuals (Fig. 11), but these morphotypes were confirmed as conspecific using 16S rRNA sequences (100% similarity). It is widely distributed across Gabon and known from Ivindo NP (Frétey and Blanc 2001), Loango NP (Burger et al. 2006), Lopé (Frétey and Blanc 2000), and Moukalaba-Doudou (Burger et al. 2004). We sequenced 16S rRNA of CAS 258243, GFMJ 1332–34, 1425, 1427–28 and confirmed their identifications using BLAST (GenBank accession: MF537671–73, MF537675, MF537680–81, MF537685–

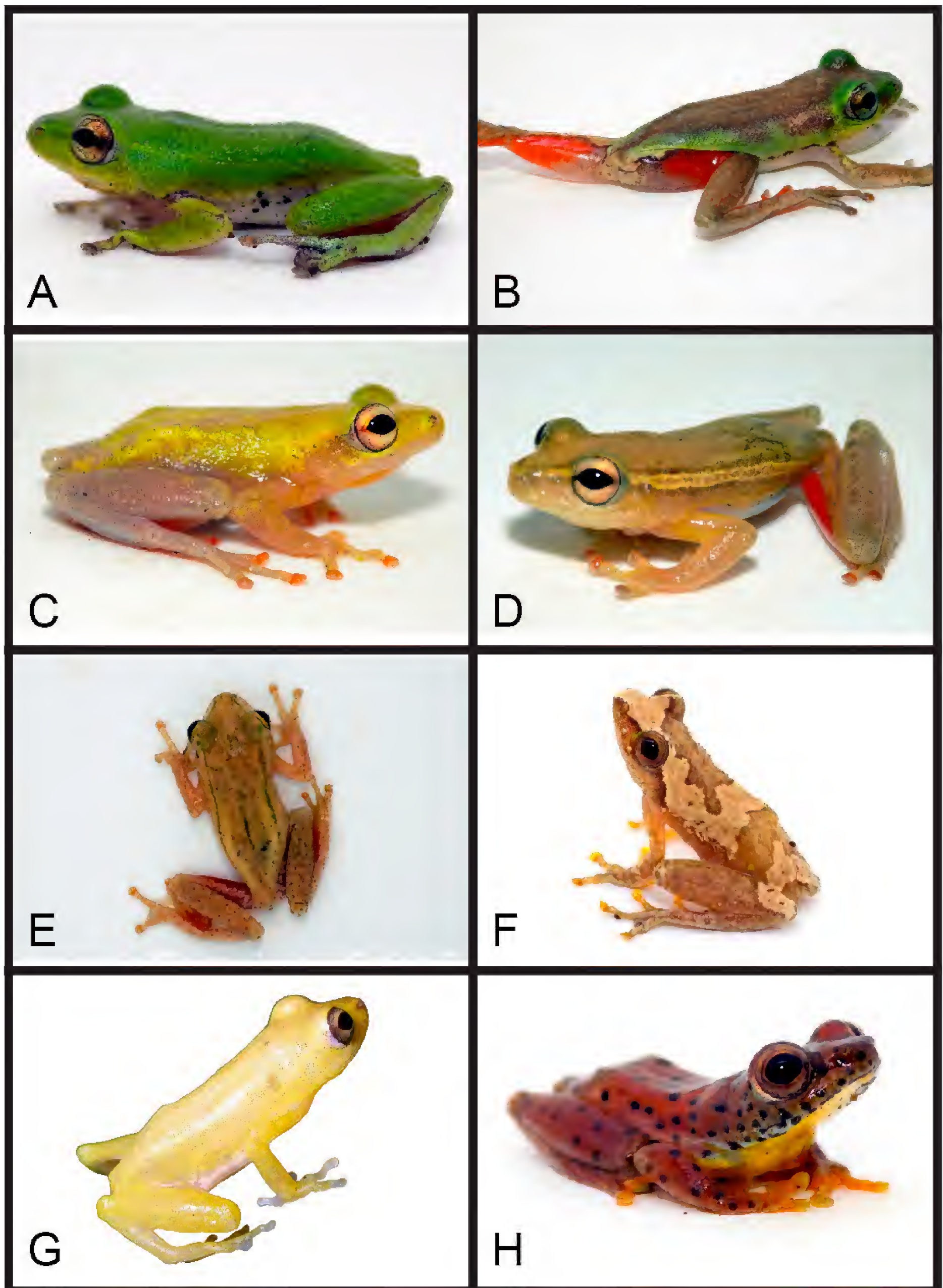


Fig. 8. *Hyperolius olivaceus* GFMJ 1381, CAS 258218, GFMJ 1181, CAS 258108 (A–D), *H. balfouri* GFMJ 1307 (E), *H. kuligae* CAS 358247 (F, G), *H. ocellatus* CAS 258177 (H).

89, MF537691; most similar to GenBank sequence GU457531; 93% identical).

***Phrynobatrachus horsti* (Rödel, Burger, Zassi-Boulou, Emmrich, Penner, and Barej 2015)**

Material: One (1) specimen. GFMJ 1332.

Comments: We encountered a single adult individual in leaf litter in the forest. Rödel et al. (2015) proposed that this species occurs in Batéké Plateau NP and that Zimkus and Larson (2013) misidentified *P. ruthbeateae*, which is endemic to Cameroon (Rödel et al. 2012). We sequenced 16S rRNA of GFMJ 1332 and confirmed their identifications using BLAST (GenBank MF537676; most similar sequence on GenBank is KR827534; 99% identical).

PYXICEPHALIDAE

***Aubria masako* (Ohler and Kazadi 1990)**

Material: One (1) specimen. Mboua: CAS 258250.

Comments: We collected a single male individual from a muddy pool adjacent to a forested stream in Mboua. Typical of this species, the individual was skittish and required multiple search events over two nights to capture successfully. *Aubria masako* is widespread in Central Africa, including specifically the Congo Basin (Ohler and Kazadi 1990). This individual was identified based on 16S rRNA (GenBank MF537692; most similar to GenBank sequence is KU560021; 99% identical).

RANIDAE

***Amnirana albolabris* (Hallowell 1856)**

Material: Nine (9) specimens. Doumaye: CAS 258140, 258146, GFMJ 1260–61. Mboua: CAS 258248–49, GFMJ 1436–37, 1504. Fig. 10G.

Comments: *Amnirana albolabris* is typically found on vegetation (~0.2–1.5 m above the ground) near still water within the forest, for example around manioc fermentation sites or elephant wallows. Individuals are easily spotted by eye-shine. The type series of this species was collected by Du Chaillu between 1855 or 1856 north of the Ogooué River in Gabon (Du Chaillu, 1861; Hallowell 1856). This species is common and widespread across Central Africa, and within Gabon it is known from Batéké Plateau NP (Zimkus and Larson 2013), Mayumba NP (USNM 2013), Loango NP (Burger et al. 2006), Lopé NP, Ivindo NP, (Frétey and Blanc 2000, 2001), and Moukalaba-Doudou (Burger et al. 2004). There are also records from Lac Oguémoué (CAS 254595–98; -1.1001600, 10.02999983), Mitone (CAS 257980; -0.641950, 10.217420), Junkville (CAS

258116; -0.062160, 11.15870), and near Ndjolé (CAS 258085; -0.193950, 10.784770).

***Amnirana amnicola* (Perret 1977)**

Material: Eight (8) specimens. Doumaye: CAS 258179–81, 258192, GFMJ 1247, 1262. Mboua: CAS 258244. Fig. 10H, Fig. 11A.

Comments: We frequently encountered this species near slow sections of forested streams on stems 1–2 m above the ground. *Amnirana amnicola* is often found in sympatry with the morphologically similar and related species *A. albolabris* from which it is distinguishable by less webbing between the toes (Perret 1977). This species is known from Crystal Mountains NP (Lötters et al. 2001), Ivindo NP (Frétey and Blanc 2001), and Moukalaba-Doudou NP (Burger et al. 2004).

***Amnirana lepus* (Andersson 1903)**

Material: Fifteen (15) specimens. Doumaye: CAS 258143–45, GFMJ 1250–52, 1255–57. Mboua: CAS 258224–26, 258279–81, GFMJ 1394. Fig. 11B, C.

Comments: This large arboreal species is commonly found along forested streams at night on vegetation 0.5–1.5 meters above the ground. A single individual was encountered on a rock in the center of a stream. *Amnirana lepus* releases a pungent sour odor when captured, which smells like vinegar. H-W Hermman observed other species kept in the same container with *A. lepus* from western Cameroon died (HWH, pers. comm.). This is possibly due to the presence of peptides (Daly et al. 2004). This species is known from Ivindo NP (Frétey and Blanc 2001), Moukalaba-Doudou (Burger et al. 2004), and near Ndjolé (CAS 258115; -0.193950, 10.784770).

RHACOPHORIDAE

***Chiromantis rufescens* (Günther 1869)**

Material: One (1) specimen. Mboua: CAS 258245. Fig. 11H.

Comments: Similar to *C. rufescens* encountered elsewhere in Gabon, we encountered this single male individual near small, temporary pools in the forest. This species is typically found on branches 1–3 m above the ground and is widespread across West and Central Africa. Within Gabon, *C. rufescens* is known from six national parks: Crystal Mountains (Bell et al. 2011), Ivindo (Frétey and Blanc 2001), Loango (Burger et al. 2006), Lopé (Frétey and Blanc 2000), Moukalaba-Doudou (Burger et al. 2004), and Batéké Plateau NP (Zimkus and Larson 2013).

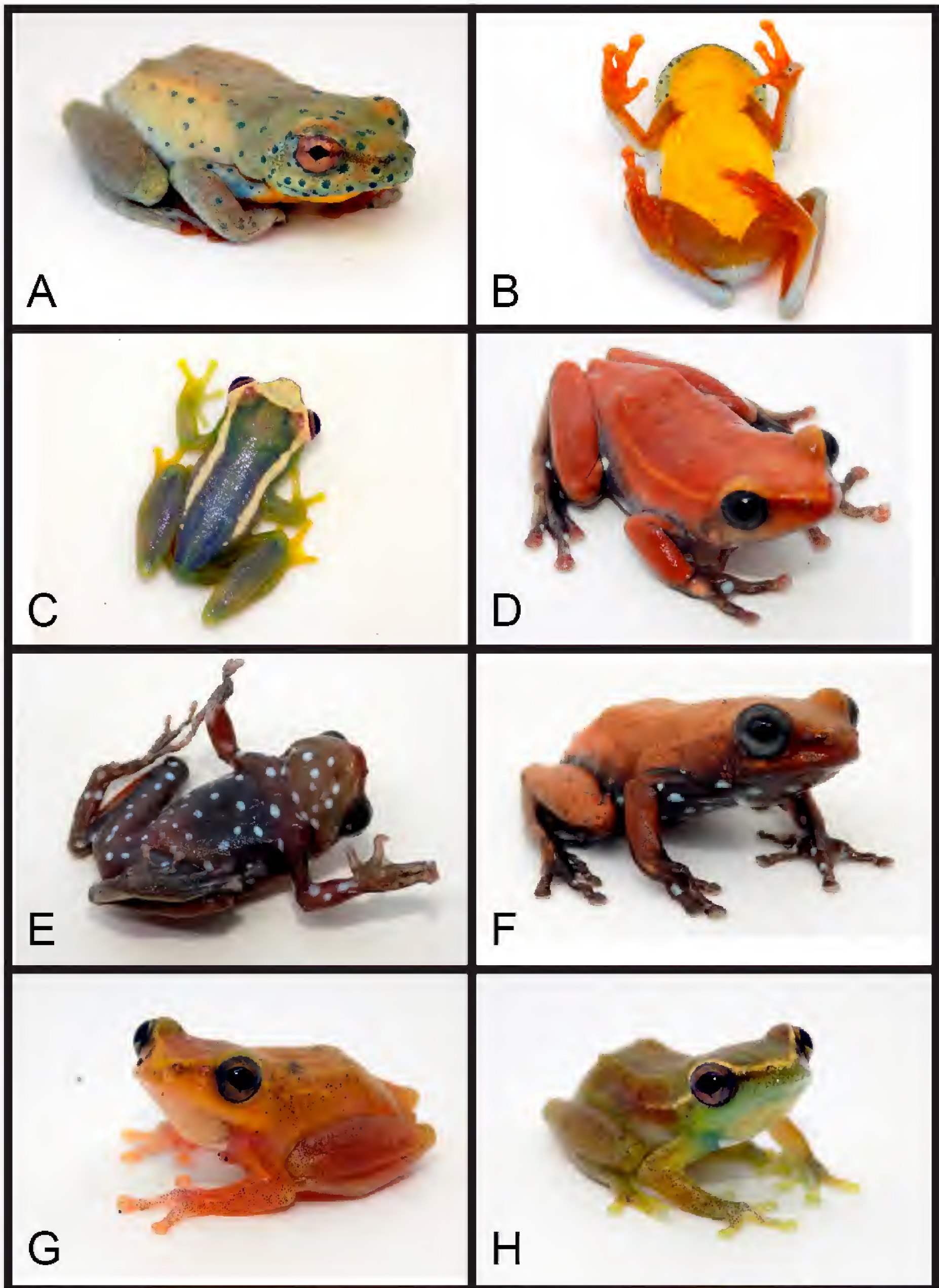


Fig. 9. *Hyperolius ocellatus* CAS 258142, GFMJ 1184 (A–C), *H. pardalis* GFMJ 1281, CAS 258204, OMNH 44797, CAS 258162 (D–H).

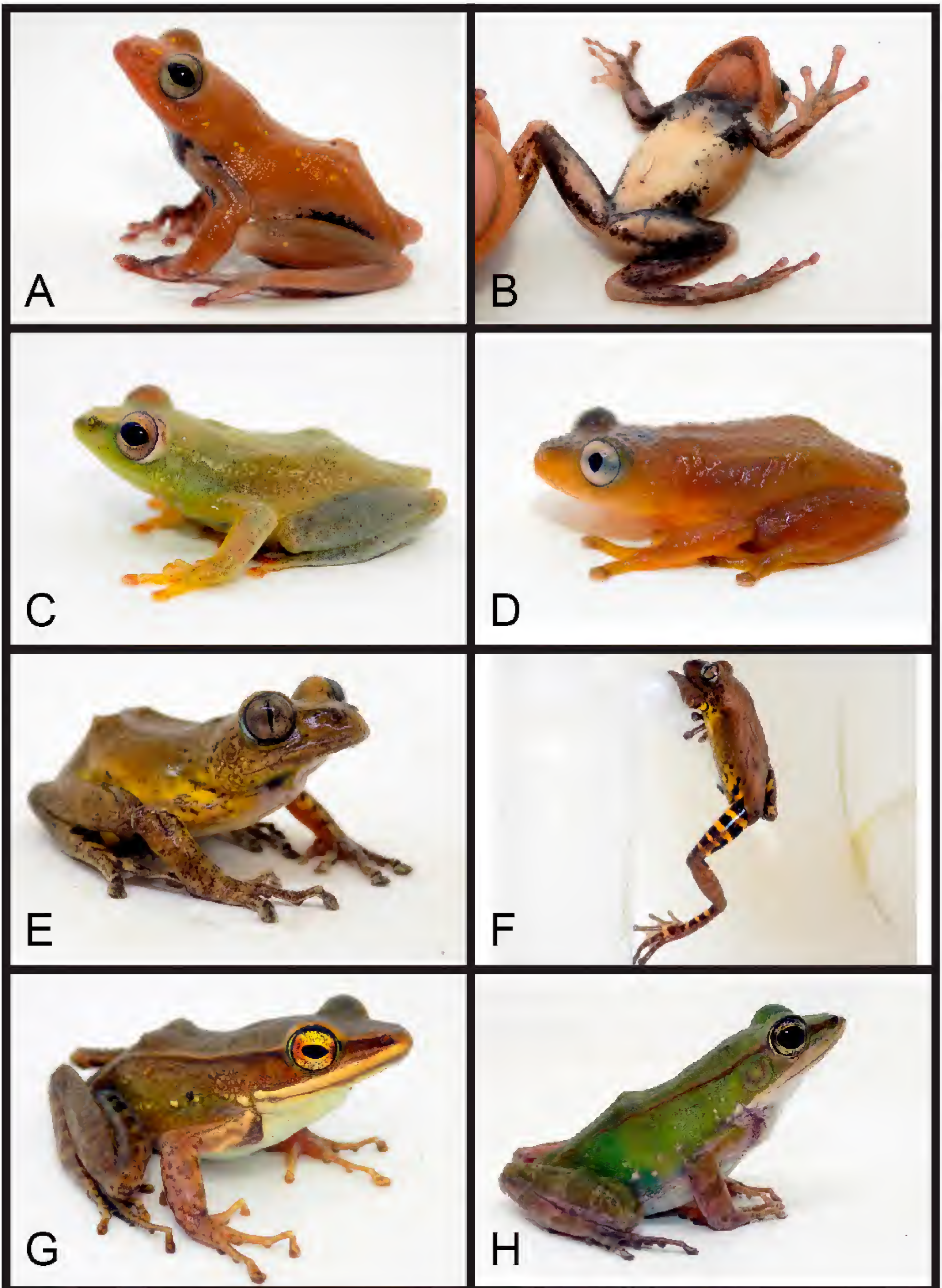


Fig. 10. *Hyperolius phantasticus* CAS 258165, 358163 (A–C), *Opisthophyllax immaculatus* CAS 258235 (D), *Phlyctimantis leonardi* CAS 258237, 2558071 (E, F), *Amnirana albolabris* ORB 291 (G), *A. amnicola* CAS 258244 (H).

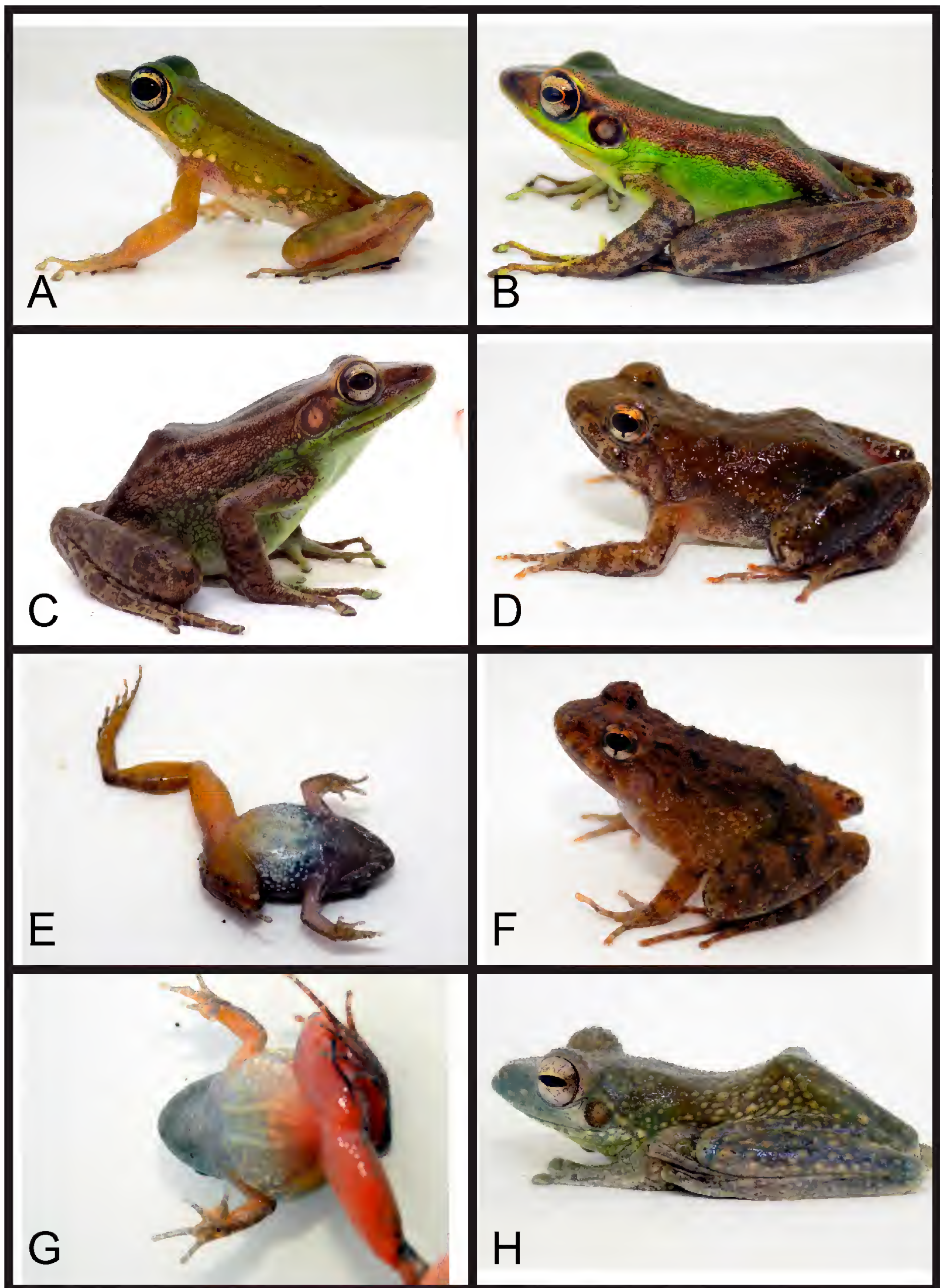


Fig. 11. *Amnirana amnicola* CAS 258224 (A) *A. lepus* CAS 258144, 258224 (B, C), *Phrynobatrachus africanus* CAS 258223, GFMJ 1389 (D, E, F, G), *Chiromantis rufescens* GFMJ 1095 (H).

Results and Discussion

Haut-Ogooué Province contains a rich diversity of amphibians but remains understudied in spite of its interesting geological history (Batéké Plateau, Chaillu Massif, Franceville Basin) and pristine savanna-forest mosaic habitat. Further inventory work will no doubt reveal more amphibian diversity. We encountered 34 amphibian species, 26 of which are new provincial records and two of which are new records for Gabon (*Afrixalus osorioi* and *Hyperolius balfouri*). These results bring the known amphibian diversity of the Haut-Ogooué province to 42 species and 98 species for Gabon. Several of our records also represent significant range extensions (>100 km from nearest known record). Our record of *Hyperolius balfouri* represents a 725-km distribution expansion south of its known distribution (nearest voucher, MHNG 1559 from Obala, Central province, Cameroon). Other range extensions include *Leptopelis calcaratus* (315 km S; nearest voucher is NCSM 77692, Ivindo NP), *ScotoBLEPS gabonicus* (275 km SE; nearest voucher NCSM 78915, Ogooué-Ivindo province), *Sclerophrys superciliaris* (320 km S, nearest locality is Makokou, Ogooué-Ivindo province), *Hyperolius kuligae* (275 km S; nearest voucher is CUMV 15570, Ogooué-Ivindo province), *H. pardalis* (310 km SE; nearest locality is Ivindo NP), and *Amnirana amnicola* (310 km SE; nearest voucher is MCZ A-139750, Ivindo NP). Laurent (1951) reported *Hyperolius steindachneri* from Franceville, Gabon. If confirmed, this record represents an 870 km extension to the north for this species and brings the total known diversity of Haut-Ogooué province to 43 species. Having not examined this specimen, we refrain from including it in the list presented here. The rarefaction curve for amphibians suggest that we are nearing the true species diversity at our sampling sites, though we anticipate that uncommon species remain to be discovered in this area (Fig. 2).

This article represents the first attempt to characterize amphibian diversity in Haut-Ogooué province in southeastern Gabon. The most amphibian-diverse province in Gabon is the Ogooué-Maritime with 77 species (Burger et al. 2004; Burger et al. 2006). Haut-Ogooué province is comparable in overall amphibian diversity to Ogooué-Ivindo, which has 46 known amphibian species (Burger et al. 2004), and Moyen-Ogooué, which has 41 species (Mocquard 1902; Pauwels 2016; specimens at the California Academy of Sciences, unpubl.). Based on GBIF data, Estuaire province has 31 known species, Ogooué-Lolo has 17, and Nyanga has 14. Other provinces have never been surveyed for amphibians (Ngounié and Woleu-Ntem). We believe that further surveys in Haut-Ogooué that focus on higher elevation sites and savannas will increase the known diversity of amphibian for this underexplored province.

Acknowledgements.—We thank the Centre National

de la Recherche Scientifique et Technologique (CENAREST, permit #AR008/15/CSAR) for providing scientific permits and the Direction de la Faune et de la Chasse for providing an export permit. For logistical support, we thank The Nature Conservancy (Marie-Claire Paiz) and the Smithsonian Institute (Lisa Korte). We are indebted to Glen Ratel and Marie Coupé for hosting us in Libreville. Thanks to Daniel M. Portik for helping identify hyperoliid species, including the new country record *A. osorioi*. We thank Jordana Abugattas for assistance with molecular genetics labwork. For assistance in the field we thank Freye Pavel for always getting us safely to our destination and keeping us fed once there. We are indebted to Chief Dipanda Guillaume and his family (Doumaye) and to Chief Michel Ngari (Mboua) for their hospitality, warmth and knowledge. Marius Burger and Olivier S.G. Pauwels provided valuable revisions on this article. Edward L. Stanley helped make Figure 1. And finally, we thank our local guides (Julien Yinga, Crepin and Blaise) that never led us astray during our nocturnal quests for frogs. This project was supported by the NSF grant (#1202609) to DCB and funding by Shell Gabon and the Smithsonian Conservation Biology Institute for ET.

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Gregory F.M. Jongsma is a Ph.D. student at the Florida Museum of Natural History at the University of Florida, USA. He received a B.S. from Acadia University (2010) and a M.S. from San Francisco State University (2014). For his dissertation he is taking a comparative phylogeographic approach to explore the diversification of frogs in Central Africa. He is seeking sponsorship from Régab and Vache Qui Rit to help sustain the team during future field work in Gabon.



Elie Tobi has been working with the Smithsonian Institution's, Gabon Biodiversity Program since 2001. He has been involved in the Monitoring and Assessment of Biodiversity in the Gamba Complex of Protected Areas. He conducted the amphibian assessment before the Loubomo-Moungagara National Road construction in South West Gabon and did recommendations to avoid and or mitigate the road construction impact on amphibian populations. He is also involved in the monitoring of Nile Crocodile nesting in the Gamba area. Elie manages an important zoological reference collection in Africa (123,000 specimens of mammals, birds, fish, reptiles, amphibians, and arthropods). He is leading environmental education and awareness programs with schools and workers of hydrocarbon companies to reduce human/animals incidents, conflicts, and impacts.

Amphibians of southeastern Gabon



Graham P. Dixon-MacCallum developed his love for herpetology over the summer before his third year at Acadia University while working for a travelling snake show in Ontario. After graduation he worked bird jobs to pay the bills but always kept an eye to the ground (and in the trees) for all things scaled or slimy. He returned officially to the world of herpetology while completing a master's degree on garter snake habitat use at the University of Victoria, British Columbia. He misses wading through elephant wallows in Gabon in search of frogs. Graham currently works in the Centre for Conservation Research at the Calgary Zoo, in Calgary, Alberta.



Abraham Bamba-Kaya has been employed since 2015 at Institut de Recherche Agronomique et Forestiere. His research interests include aquatic biodiversity, conservation, and investigating amphibian declines. He has participated in expeditions of assessments and surveys of amphibians in various localities in Gabon.



Jean-Aimé Yoga is a researcher at the National Center for Scientific and Technological Research (CENAREST) of Gabon. He has a special interest in herpetology and has been involved in several projects since 2005. He helped with monitoring and assessing biodiversity at the Rabi site for amphibians and reptiles (Smithsonian Institution Project). He was the first to collect *Ramphotyplops braminus* (Snakes: Tylopidae) from Gabon.



Jean-Daniel Mbega is the head researcher at the Laboratory of Hydrobiology and Oenology of the National Center based at Institut de Recherches Agronomiques et Forestières (IRAF/CENAREST). Jean Daniel has made several important contributions to our knowledge about the freshwater fish of Gabon. In 2008, the National Assembly of Gabon presented Dr. Mbega the gold medal for his published *Identification Guide of the Fishes of the Lower Ogooué Basin*.



Jean Hervé Mvé Beh is a researcher at the Laboratory of Hydrobiology and Oenology of the National Center for Scientific and Technological Research (CENAREST) of Gabon. There are nearly twenty-two experiments on projects on freshwater fish in Gabon and brackish fish in Gabon. He participates in several projects with a focus on taxonomy, biology, and conservation. He participated in the development of the IUCN report on marine fish in the eastern Atlantic. Jean Hervé is currently working on a project on the role of mangroves in the Akanda National Park as a nursery for species of commercial interest landed by artisanal fisheries. It has just contributed to a project financed by TNC Gabon and to the study of the baselines of the fish populations of the sites proposed for the hydropower project. Jean Hervé is a member of the scientific society Gilbert.



Andi Emrich fostered a love for amphibians as a small child in Canada but re-invigorated the spark after working with Greg Jongsma on his research in Ecuador in 2007. After moving to San Francisco, Andi got involved at the California Academy of Sciences, volunteering her time in the mammalogy collections. She now works for the Florida Organic Growers in Gainesville, Florida but hopes to get back in the field on another amphibian adventure soon.



David C. Blackburn is the Associate Curator of Herpetology at the Florida Museum of Natural History at the University of Florida, USA. He received a BA from the University of Chicago (2001) and a Ph.D. from Harvard University (2008). His research focuses on the diversity and evolution of frogs. He hopes to one day see the following strange frogs alive in the field: *Calyptocephalella gayi*, *Conraua beccarii*, *Myobatrachus gouldii*, and *Triprrion petastatus*.



Preliminary herpetological survey of Ngonye Falls and surrounding regions in south-western Zambia

^{1,2,*}Darren W. Pietersen, ³Errol W. Pietersen, and ^{4,5}Werner Conradie

¹Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028, SOUTH AFRICA ²Research Associate, Herpetology Section, Department of Vertebrates, Ditsong National Museum of Natural History, P.O. Box 413, Pretoria, 0001, SOUTH AFRICA ³P.O. Box 1514, Hoedspruit, 1380, SOUTH AFRICA ⁴Port Elizabeth Museum (Bayworld), P.O. Box 13147, Humewood, 6013, SOUTH AFRICA ⁵School of Natural Resource Management, George Campus, Nelson Mandela Metropolitan University, George, SOUTH AFRICA

Abstract.—The herpetofauna of Zambia has been relatively well-studied, although most surveys were conducted decades ago. In western Zambia in particular, surveys were largely restricted to a few centers, particularly those along the Zambezi River. We here report on the herpetofauna of the Ngonye Falls and surrounding regions in south-western Zambia. We recorded 18 amphibian, one crocodile, two chelonian, 22 lizard, and 19 snake species, although a number of additional species are expected to occur in the region based on their known distribution and habitat preferences. We also provide three new reptile country records for Zambia (Long-tailed Worm Lizard, *Dalophia longicauda*, Anchieta's Worm Lizard, *Monopeltis anchietae*, and Zambezi Rough-scaled Lizard, *Ichnotropis grandiceps*), and report on the second specimen of Schmitz's Legless Skink, *Acontias schmitzi*, a species described in 2012 and until now known only from the holotype. This record also represents a 140 km southward range extension for the species.

Keywords. Sioma Ngwezi National Park, Barotseland, Western Province, Africa, distribution, reptiles, lizards, amphibians

Citation: Pietersen DW, Pietersen EW, Conradie W. 2017. Preliminary herpetological survey of Ngonye Falls and surrounding regions in south-western Zambia. *Amphibian & Reptile Conservation* 11(1) [Special Section]: 24–43 (e148).

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Received: 19 November 2017; **Accepted:** 13 December 2017; **Published:** 31 December 2017

Introduction

Despite the herpetofauna of Zambia being relatively well-studied (e.g., Broadley 1971a), large areas of the country remain poorly surveyed (Broadley 2000a; Poynton and Broadley 1985a). Most collecting was conducted prior to 1970, although important subsequent contributions were made by Broadley (1991a,b, 2000a), Branch and Haagner (1993), Haagner et al. (2000), Chansa and Wagner (2006) and Wagner et al. (2012a,b,c, 2013). The last systematic review of Zambia's herpetofauna was undertaken by Broadley (1971a), at which time there were 65 amphibian, two crocodile, nine chelonian, 54 lizard, and 75 snake species recorded. The amphibians of Zambia have been dealt with in detail by Poynton and Broadley (1985a,b, 1987, 1988, 1991a), while snakes were covered by Broadley et al. (2003). Since these publications a number of taxonomic changes have occurred, new species described (Broadley 2014; Wagner et al. 2012a,b), and species recorded from Zambia

for the first time (Broadley and van Daele 2003; Wagner et al. 2013). Currently the Zambian herpetofauna comprises 85 amphibian, two crocodile, 10 chelonian, 75 lizard, and 91 snake species (AmphibiaWeb 2016; Broadley 1971a; Broadley and van Daele 2003; Uetz et al. 2017). Of these, 35 amphibian, one crocodile, three chelonian, 27 lizard, and 39 snake species are known from the Barotse Floodplains and surroundings (Broadley 2000a).

Barotseland lies at the junction of three broad zoogeographic zones, viz. mesic Angolan/Congolian Zone, arid Kalahari Zone, and the East African coastal zone, and the region is thus expected to support high herpetofaunal diversity (Timberlake 2000). Western Zambia remains one of the neglected regions of Zambia from a biodiversity perspective, although it received attention during the cross-border Zambezi Basin Wetland survey conducted in the late 1990s (Broadley 2000a; Channing 2000; Timberlake 2000). Targeted surveys of the Barotse Floodplains led to the description of a new frog species, *Hemisus barotseensis* (Channing and Broadley 2002).

Correspondence. *pietersen.darren@gmail.com

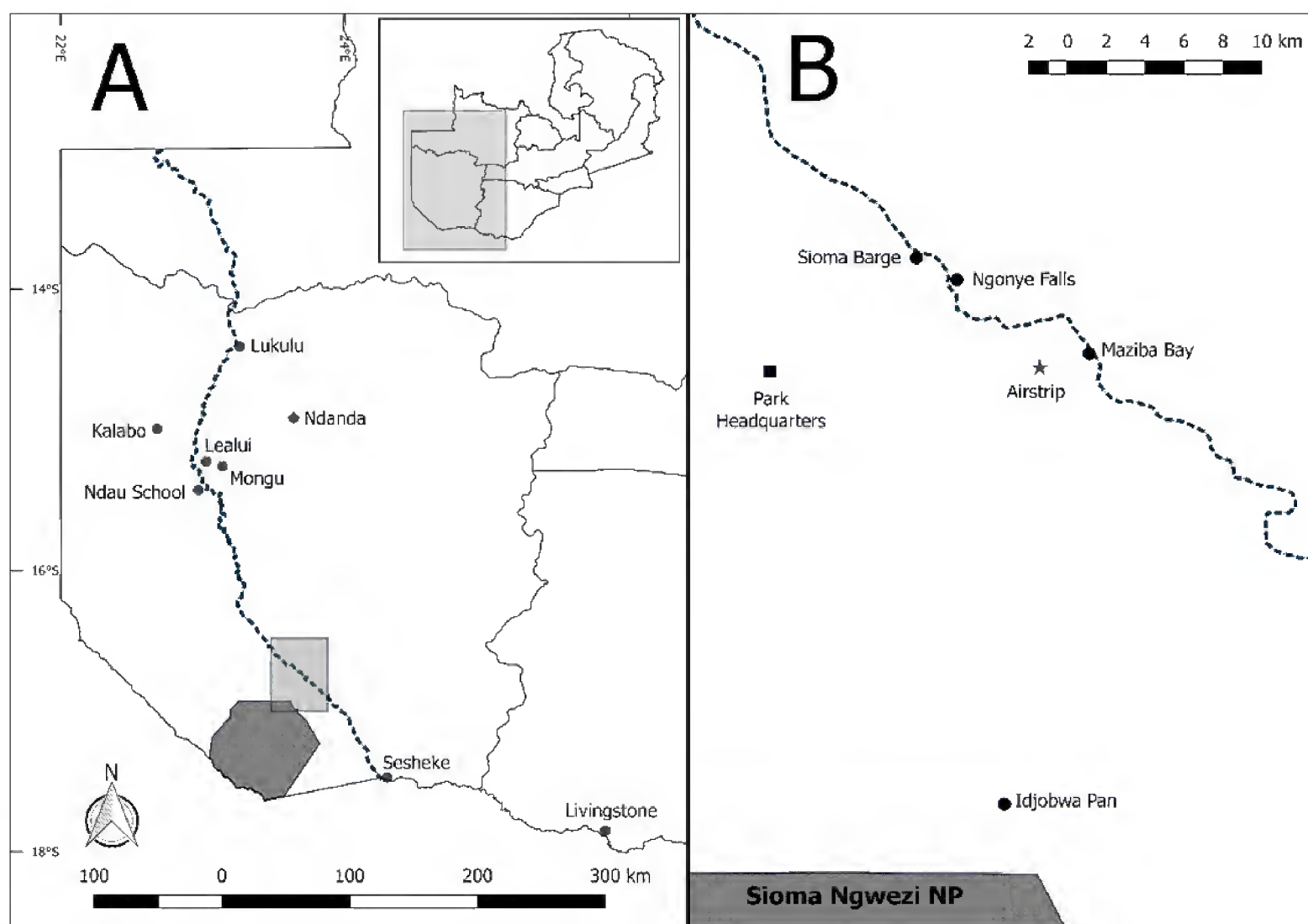


Fig. 1. (A) Map of western Zambia indicating major localities mentioned in the text, including Sioma Ngwezi National Park (dark grey polygon). The light grey rectangle indicates the study site, and the dotted line indicates the Zambezi River. Inset: Map of Zambia indicating the enlarged region. **(B)** Enlarged study site with locality names. The Ngonye Falls campsite and visitor's center are both within 1 km of Ngonye Falls and are therefore not indicated on the map.

Prior to this, small collections from western Zambia were reported on by Roux (1907), Angel (1920, 1921, 1922) and Broadley (1968a, 1971a), which led to the descriptions of various snakes and lizards from the region, including *Dalophia ellenbergeri* (Angel, 1920); *Tetradactylus ellenbergeri* (Angel, 1922); *Typhlacontias gracilis* Roux, 1907; *T. rohani* Angel, 1923; *Acontias jappi* (Broadley, 1968); *Amblyodipsas ventrimaculata* (Roux, 1907); *Crotaphopeltis barotseensis* Broadley, 1968 and *Zygaspis nigra* Broadley and Gans, 1969.

We had the opportunity over a period of three-and-a-half-years to document the herpetofauna of the Ngonye Falls region in western Zambia, and present here an initial inventory.

Methods

Study area

The Ngonye Falls are located in the Western Province in south-western Zambia (Fig. 1). A tourist attraction in its own right (Fig. 2a), it is also the location of the administrative headquarters of Sioma Ngwezi National Park, which is situated to the south-west. The Ngonye Falls form a northern extension of the Sioma Ngwezi National Park, and as such the falls and a small area surrounding it are afforded official protection. The vegetation falls into the Flora Zambesiaca bioregion and is dominated by *Baikiaea* woodland on deep Kalahari soils, although intense settlement and subsistence agriculture prevail in

the vicinity of the Zambezi River. Rupicolous habitat is restricted to the immediate vicinity of the Zambezi River. Geographical coordinates for the main localities mentioned in the species accounts are presented in Table 1.

Data collection

One of us (EWP) was stationed permanently at Ngonye Falls from 8 February 2013 to 30 August 2016. During this time reptiles and amphibians were recorded incidentally, with some active searching. Herpetofauna were actively searched for on the western bank of the Zambezi River by DWP, John Davies, and EWP from 21 to 28 April 2013, on the eastern shore by WC from 17 to 27 October 2015, and Roger Bills from 7 to 11 October 2017. Voucher specimens were not collected in the early stages of this survey due to the lack of collecting permits, but in these instances photographic records were obtained, as has also been done in other surveys and regional works (e.g., Tuberville et al. 2005; Gooley et al. 2011; Bates et al. 2014).

All surveys involved opportunistic visual encounters. Diurnal surveys involved actively searching specific microhabitats, particularly beneath rocks and decaying logs. Nocturnal surveys for amphibians were undertaken in wetlands and surrounding woodland. Two standard Y-shape trap arrays were deployed on the eastern bank of the Zambezi River in October 2015, with each array consisting of three drift fences (10 m long and 50 cm high), with four pitfall traps (one at the center and at each fence



Fig. 2. Selection of amphibians and reptiles photographed in the vicinity of Ngonye Falls, south-western Zambia. (A) View of Ngonye Falls from the eastern side of the Zambezi River. (B) Mapacha Grass Frog (*Ptychadena* cf. *mapacha*, VMUS 5990), Sioma Ngwezi National Park Headquarters. (C) Long-tailed Worm Lizard (*Dalophia longicauda*), Sioma Ngwezi National Park Headquarters. (D) Zambezi Rough-scaled Lizard (*Ichnotropis grandiceps*, TM 86237), Sioma Ngwezi National Park Headquarters. (E) Barotse Blind Legless Skink (*Acontias jappi*, TM 86232), Sioma Ngwezi National Park Headquarters. (F) Eastern Black-lined Plated Lizard (*Gerrhosaurus intermedius*), Sioma Ngwezi National Park Headquarters.

tip, respectively) and six one-way funnel traps placed on opposite sides of the fences in the center of each arm.

Specimens retained for subsequent study were humanely euthanized by injecting tricaine methanesulfonate (MS222) solution into the intracoelomic cavity for reptiles (Conroy et al. 2009), and submerging frogs in a MS222 solution, after which they were formalin-fixed for 48 hours and then transferred to alcohol for long-term storage. Prior to fixing, tissue samples (either liver or muscle) were preserved in 96% ethanol for use in genetic analyses. Voucher specimens (Appendix 1) are held in the herpetological collections of the Port Elizabeth Museum (PEM), Ditsong National Museum of Natural History, Pretoria (TM), and South African Aquatic Biodiversity Institute, Grahamstown (SAIAB). Reptile and amphibian photographic records were submitted to

the Animal Demography Unit Virtual Museum (Available: <http://vmus.adu.org.za>) on the platforms ReptileMAP and FrogMAP, respectively. Ventral scales were counted from the first scale posterior to the mental up to (but excluding) the cloacal shield. Subcaudal scales were counted from the first scale posterior to the cloacal shield up to, but excluding, the terminal scale. For amphisbaenids, dorsal annuli were counted along the dorsal midline from the first whole annulus posterior to the head up to the last annulus anterior to the cloacal shield. Caudal annuli were counted along the dorsal midline starting at the first complete annulus posterior to the cloacal shield, up to (but excluding) the terminal pad.

Relevant field guides (Branch 1998; Broadley 1983; Broadley et al. 2003; Channing 2001; du Preez and Carruthers 2009) were used for species identification.

Table 1. Coordinates for the main localities at and around Ngonye Falls and Sioma Ngwezi National Park, as mentioned in the text.

Locality	Latitude	Longitude
Ngonye Falls airstrip	16° 41' 40" S	23° 36' 49" E
Ngonye Falls campsite	16° 39' 41" S	23° 34' 23" E
Park Headquarters	16° 40' 08" S	23° 34' 03" E
Visitor's center	16° 39' 24" S	23° 34' 11" E
Maziba Bay	16° 41' 16" S	23° 38' 12" E
East bank of Zambezi River opposite Ngonye Falls	16° 39' 20" S	23° 34' 35" E
West bank of Zambezi River opposite Ngonye Falls	16° 39' 11" S	23° 34' 14" E
Idjobwa Pan	16° 53' 48" S	23° 35' 50" E
Sioma Barge	16° 38' 35" S	23° 33' 25" E

Nomenclature was based on established online databases (amphibians: Frost 2016; reptiles: Uetz et al. 2017), updated where appropriate. Vernacular names follow du Preez and Carruthers (2009) for amphibians and Branch (1998) for reptiles, updated from Frost (2016) and Uetz et al. (2017) for those taxa not covered by these guides. No regional conservation assessment has been undertaken for Zambian amphibians and reptiles as yet, but where global conservation assessments are available (IUCN 2017) these are noted. Endemic (defined as species with ranges restricted to Zambia) and near-endemic species (>90% of distribution within Zambia) are also indicated.

Species accounts

Amphibia

Breviceptidae

Breviceps adspersus adspersus Peters, 1882

Bushveld Rain Frog

Photograph: VMUS 5982

Individuals were photographed in Ngonye Falls campsite, and were heard calling from this area, from the visitor's center and from the vicinity of Park Headquarters. This species is distinguished from *B. poweri* on the basis of call, having a series of pale paravertebral and dorsolateral patches, absence of a continuous pale line from the upper lip to the forearm, and having a less intense dark throat that is medially divided by a white line (du Preez and Carruthers 2009; Poynton and Broadley 1985a, 1991). In Zambia, this species has been collected only in the vicinity of Kalabo, about 200 km to the NNW (Channing 2001; Poynton and Broadley 1985a, 1991).

Breviceps poweri Parker, 1934

Power's Rain Frog

Photograph: VMUS 5983

This species was often heard, and photographed, in the vicinity of Ngonye Falls campsite, visitor's center and Park Headquarters. It is distinguished from *B. adspersus* on the basis of call, absence of paravertebral patches (usually present in *B. adspersus*); presence of a pale patch above the vent (usually absent in *B. adspersus*); uniformly dark throat (usually mottled in *B. adspersus*); continuous pale band from upper lip to forearm; and presence of a short, dark band between the nostrils and mouth (usually not well developed in *B. adspersus*; du Preez and Carruthers 2009; Poynton and Broadley 1985a, 1991). This record extends the distribution of the species by about 200 km SSE from Kalabo.

Bufonidae

Poyntonophrynus fenoulheti (Hewitt and Methuen, 1913)

Northern Pygmy Toad

Photograph: VMUS 5989

A single individual was photographed at Ngonye Falls campsite. It was distinguished from *P. kavangensis* on the basis of the tympanum being distinctly visible, and the presence of small tubercles on the dorsal surface of the snout (du Preez and Carruthers 2009). The only previous Zambian records are from the northern shore of Lake Kariba and the Zambian bank of the Victoria Falls (Broadley 1971a; Channing 2001; Poynton and Broadley 1988, 1991), while the nearest locality is Katima Mulilo in the Zambezi Region of Namibia (Channing 2001; Poynton and Broadley 1991). This record extends the known range of this species 110 km north-west.

Schismaderma carens (Smith, 1848)

Red Toad

Material: SAIAB 205361, 205631

Photographs: VMUS 5992, 5993

Individuals were recorded at Ngonye Falls campsite,

at the airstrip, and on the eastern banks of the Zambezi River below Ngonye Falls. This species was previously recorded at Livingstone and Kalabo in Western Province (Broadley 1971a; Channing 2001; Poynton and Broadley 1988, 1991).

Sclerophrys gutturalis (Power, 1927)

Guttural Toad

Photograph: VMUS 5994

Seen at Ngonye Falls campsite, and common throughout Zambia (Broadley 1971a; Channing 2001; Poynton and Broadley 1988, 1991).

Sclerophrys poweri (Hewitt, 1935)

Western Olive Toad

Material: SAIAB 205356

Photographs: VMUS 5996, 5997

Observed and photographed at Ngonye Falls campsite, and collected on the eastern banks of the Zambezi River below Ngonye Falls. These records extend the known distribution of this species about 110 km north-west from the nearest records at Sesheke (Channing 2001; Poynton and Broadley 1988, 1991), although Broadley (2000a) records it from the “Barotse floodplains.”

Sclerophrys pusilla (Hallowell, 1855)

Flat-backed Toad

Material: PEM A11719, 11720; SAIAB 205360

Photographs: VMUS 5995, 5998

Individuals were seen at Ngonye Falls campsite, as well as on the eastern bank of the Zambezi River opposite Ngonye Falls. In Western Province it has been collected only at Kalabo (Broadley 1971a; Channing 2001; Poynton and Broadley 1991).

Hemisotidae

Hemisus marmoratus (Peters, 1854)

Mottled Shovel-nosed Frog

Photograph: VMUS 5985

One individual was photographed in Ngonye Falls campsite. It was distinguished from *H. guineensis* on the basis of coloration (dorsum mottled light and dark in *H. marmoratus* and dark with small yellow, orange or white spots in *H. guineensis*; Channing 2001; du Preez and Carruthers 2009). It was distinguished from *H. barotseensis* by having the upper eyelid length exceeding the eye-nostril distance (Channing 2001; Channing and Broadley 2002). The only previous record of this species in Western Province is at Livingstone, although also

recorded from Katima Mulilo in Namibia (Poynton and Broadley 1985a, 1991).

Hyperoliidae

Hyperolius angolensis Steindachner, 1867

Angolan Reed Frog

Photograph: VMUS 5986

A single individual, which we tentatively refer to *H. angolensis*, was photographed at Ngonye Falls campsite. This species is likely to be more common and is probably found in pans and other temporary wetlands in Sioma Ngwezi National Park, as well as other wetlands associated with the Zambezi and Cuando Rivers. The only previous Zambian records are from the western shore of the Upper Zambezi at Sandaula Plain, Kalabo and Kalenga (Broadley 1971a; Poynton and Broadley 1987), although it is fairly widespread in the Okavango Swamps of Botswana and the Zambezi Region of northern Namibia (Poynton and Broadley 1987, 1991) as well as south-eastern Angola (Conradie et al. 2016). This record partially bridges the gap between the Namibian and Upper Zambezi records. The taxonomic status of this species remains unresolved and it is considered part of the larger unresolved *H. parallelus* Günther, 1858 group which is widespread across Angola and adjacent countries (Frost 2016). Many regional color patterns exist, the specimen from Ngonye Falls conforms best to that of *H. angolensis* (fide Schiøtz 1999).

Phrynobatrachidae

Phrynobatrachus natalensis (Smith, 1849)

Snoring Puddle Frog

Material: SAIAB 205351

Photograph: VMUS 5987

This species was recorded on the west bank of the Zambezi River in the vicinity of Ngonye Falls and campsite, as well as on the eastern bank of the Zambezi River opposite Ngonye Falls. It is widespread in Zambia (Broadley 1971a; Channing 2001; Poynton and Broadley 1985b, 1991).

Phrynobatrachus parvulus (Boulenger, 1905)

Small Puddle Frog

Photograph: VMUS 5988

An individual was photographed at Ngonye Falls campsite. The only previous record for Western Province is Ngambwe Rapids, about 90 km to the south-east (Channing 2001; Poynton and Broadley 1985b, 1991).

Pipidae

Xenopus muelleri (Peters, 1844)

Müller's Platanna

Material: SAIAB 202357

One male and one female were collected in sympatry with *X. poweri* in an eastern tributary (16°39'07"S, 23°37'43"E) flowing into the Zambezi River. Conradie et al. (2016) also recorded these two species in sympatry in south-eastern Angola.

Xenopus poweri Hewitt, 1927

Power's Platanna

Material: SAIAB 202355

One male and one female were collected in sympatry with *X. muelleri* in an eastern tributary of the Zambezi River (see above).

Ptychadenidae

Ptychadena cf. *mapacha* Channing, 1993

Mapacha Grass Frog

Material: TM 86255

Photograph: VMUS 5990

One individual (TM 86255) was collected at Park Headquarters after being killed by a vehicle, while a second individual (VMUS 5990, Fig. 2b) was photographed at the same site. Individuals are provisionally assigned to this species based on external morphology and coloration, however molecular analyses and/or call recordings are required to unequivocally confirm these identifications. For many years this species was only known from the type locality, viz. Mapacha Airfield and the area surrounding Katima Mulilo in the Zambezi Region of Namibia (Channing 1993; du Preez and Carruthers 2009), although expected to occur in south-western Zambia, south-eastern Angola and northern Botswana as well (Channing 2001). Haacke (1999) collected four individuals along the Ojmatako River about 80 km east of Rundu (this record has largely been overlooked in the literature), while most recently Ceriaco et al. (2016) recorded it from Rundu District in Kavango-East Region, northern Namibia. Conradie et al. (2016) collected a series of *Ptychadena* at Jamba in neighboring south-eastern Angola which they provisionally assigned to *P.* cf. *mosambica*, although noting that their specimens may be referable to *P. mapacha*. The records reported here are the first for Zambia and the first outside Namibia. These records extend the known distribution of this Data Deficient species 120 km NNW into Zambia, and 320 km NNE into south-eastern Angola, which could have positive conservation implications (IUCN SSC Amphibian Specialist Group, SA-FRoG 2017).

Ptychadena oxyrhynchus (Smith, 1849)

Sharp-nosed Grass Frog

Material: SAIAB 205353, 205354

Photograph: VMUS 5991

An individual was photographed at Ngonye Falls campsite, and individuals were also heard calling from the western bank of the Zambezi River in the vicinity of Ngonye Falls. Additional material was collected from the eastern bank of the Zambezi River below Ngonye Falls. This species is widespread in Zambia (Broadley 1971a; Channing 2001), although the only other published record for Western Province is Sesheke (Channing 2001; Poynton and Broadley 1985b, 1991), 110 km to the south-east.

Ptychadena subpunctata (Bocage, 1866)

Speckled-bellied Grass Frog

Material: PEM A11717, 11718; SAIAB 205358, 205365

This species was recorded from the eastern shore of the Zambezi River, opposite Ngonye Falls. It is widespread in Zambia, including Upper Zambezi Region (Broadley 1971a; Channing 2001; Poynton and Broadley 1985b, 1991).

Pyxicephalidae

Tomopterna cf. *cryptotis* (Boulenger, 1907)

Tremolo Sand Frog

Material: SAIAB 205362

Photographs: VMUS 5999, 6000

Recorded at Ngonye Falls campsite and on the eastern banks of the Zambezi River below Ngonye Falls. Previously collected in Western Province at Kalabo, Sandaula Plain and Sesheke (Channing 2001; Poynton and Broadley 1985b, 1991), and our records partially fill the gap between these localities. We provisionally assign our records to *T. cryptotis* based on distribution, but note that species delineation in this genus is problematic when based solely on external morphology, and these specimens may in fact refer to the similar Tandy's Sand Frog *T. tandyi* Channing and Bogart, 1996.

Rhacophoridae

Chiromantis xerampelina Peters, 1854

Southern Foam Nest Frog

Photograph: VMUS 5984

An individual was photographed at the airstrip. Although widespread in Zambia, the only previous records from Western Province are Sesheke and Lukulu (Broadley 1971a; Channing 2001; Poynton and Broadley 1987, 1991).

Reptilia

Squamata

Sauria

Agamidae

Agama armata Peters, 1855

Peter's Ground Agama

Material: PEM R22017, 22018

Photographs: VMUS 158866–158869

Recorded regularly at Ngonye Falls campsite and visitor's center, and the vicinity of the airstrip. It is widespread in Zambia (Broadley 1971a).

Amphisbaenidae

Dalophia longicauda (Werner, 1915)

Long-tailed Worm Lizard

Photograph: VMUS 163523

A single individual (Fig. 2c) was found on the soil surface in the vicinity of Park Headquarters. It is uniform flesh-pink in color with 326 dorsal and 37 caudal annuli, with the tail ending in a calloused pad. There is no constricted caudal autotomy site, and the dorsal caudal annuli form a “herring-bone” pattern. The cephalic shield consists of a single large plate, with lateral sulci. This individual is distinguished from *D. angolensis* and *D. ellenbergeri* by the absence of a constricted caudal autotomy site (Broadley et al. 1976). It is further distinguished from both *D. angolensis* and *D. pistillum* by the high subcaudal counts (usually 20–27 caudal annuli in *D. angolensis* and 19–33 in *D. pistillum*). It is distinguished from *D. angolensis*, *D. ellenbergeri*, and *D. pistillum* by the dorsal caudal annuli forming a “herring-bone” pattern (Broadley et al. 1976). This represents the first record of this fossorial species in Zambia (Branch 1998; Broadley 1971a; Broadley et al. 1976; Uetz et al. 2017).

Dalophia pistillum (Boettger, 1895)

Blunt-tailed Worm Lizard

Material: PEM R22925

A single individual was collected on the eastern bank of the Zambezi River below Ngonye Falls. This species is distinguished from other *Dalophia* in Zambia by the absence of a constricted caudal autotomy annulus (present in *D. angolensis* and *D. ellenbergeri*), lower numbers of caudal annuli (27 versus 33–42 in *D. longicauda*), and absence of a “herring-bone” pattern on the dorsal caudal annuli (present in *D. longicauda*; Broadley et al. 1976). It is fairly widespread in southern and western Zambia, although records are sparse due to its predominantly fossorial habits (Broadley et al. 1976).

Monopeltis anchietae (Bocage, 1873)

Anchieta's Worm Lizard

Material: TM 86250

One individual (Fig. 3a,b) was found beneath an elephant carcass at Idjobwa Pan in the buffer zone to the north of Sioma Ngwezi National Park, while a juvenile (TM 86250) was unearthed during construction at Park Headquarters. Members of the genus *Monopeltis* generally inhabit deep Kalahari sands, only coming to the surface after their burrows have been flooded by heavy rains (Branch 1998, DWP pers. obs.). These are the first records of this species in Zambia. Previously known from northern Botswana, northern Namibia and southern Angola (Broadley 1971a; Broadley et al. 1976; Uetz et al. 2017).

Zygaspis nigra Broadley and Gans, 1969

Black Round-headed Worm Lizard

Material: TM 86209

Photograph: VMUS 158938

This small fossorial species was collected with *Z. quadri-frons* in Baikiaea woodland at the airstrip. Known from Zambia, Angola, and northern Namibia, with most Zambian specimens collected at Kalabo, the type locality, with a subsequent record from Ndau School (ca. 25 km south-west of Mongu on the western side of the Zambezi River; Broadley 2000a). The new record partially fills the gap between the Ndau School, eastern Angola and Namibia records.

Zygaspis quadri-frons (Peters, 1862)

Kalahari Round-headed Worm Lizard

Material: TM 86208

Photograph: VMUS 158939

One individual was found in Baikiaea woodland at the airstrip, while a second was found near Ngonye Falls campsite. This species is probably quite common and widespread throughout the area (see also Broadley 1971a), being overlooked due to its fossorial nature.

Chamaeleonidae

Chamaeleo dilepis Leach, 1819

Flap-neck Chameleon

Photographs: VMUS 158873, 158876, 158877

Recorded regularly around Park Headquarters, visitor's center, and Ngonye Falls campsite.

Gekkonidae

Pachydactylus wahlbergii wahlbergii (Peters, 1869)

Kalahari Thick-toed Gecko

Photographs: VMUS 163521, 163522

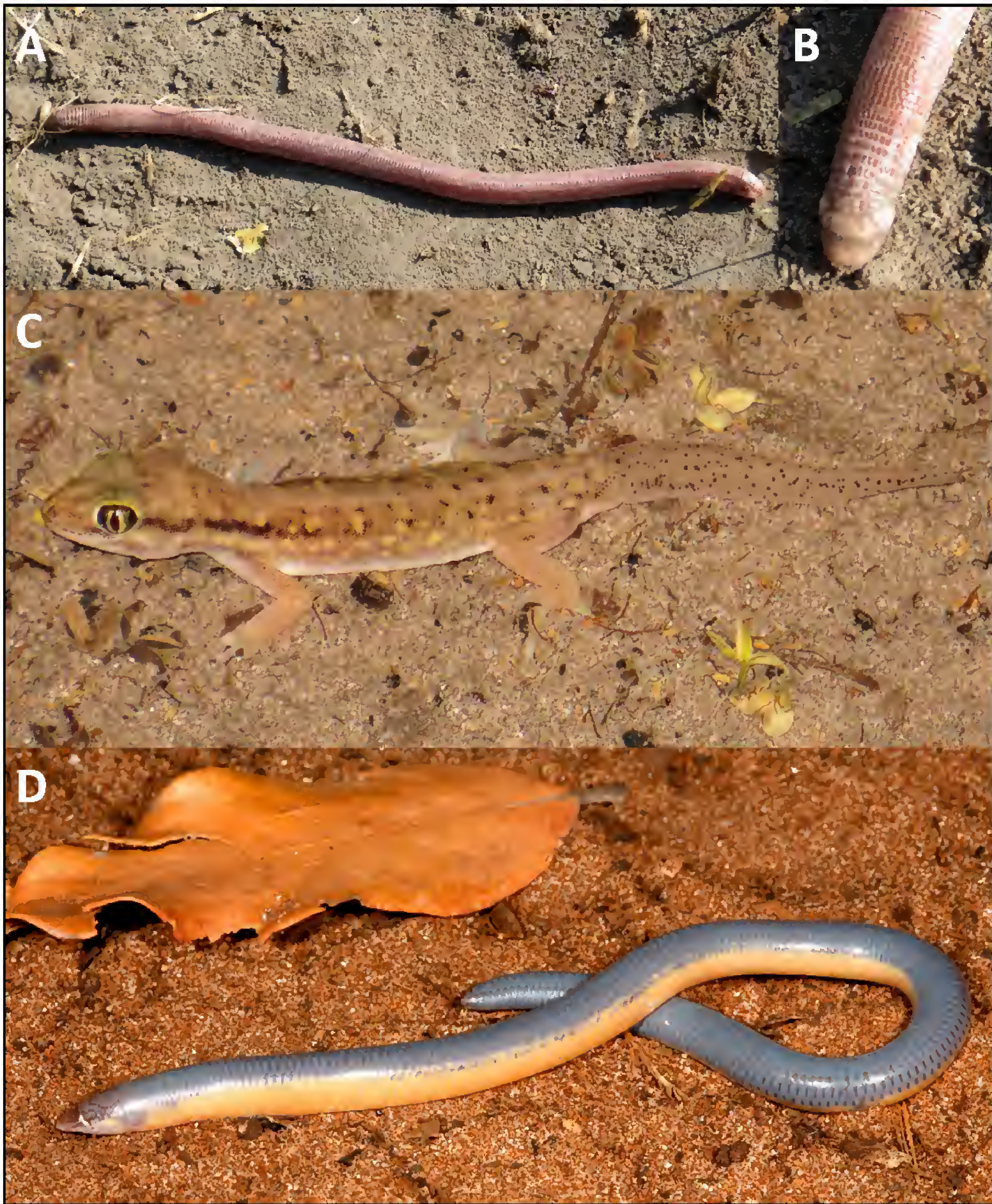


Fig. 3. Selection of reptiles photographed in the vicinity of Sioma Ngwezi National Park and Ngonye Falls in south-western Zambia. (A) Anchieta's Worm Lizard (*Monopeltis anchietae*), Idjobwa Pan, northern buffer zone of Sioma Ngwezi National Park and (B) close-up of the head, showing the double head shields. (C) Wahlberg's Kalahari Gecko (*Pachydactylus wahlbergii wahlbergii*), Ngonye Falls campsite. (D) Schmitz's Blind Legless Skink (*Acontias schmitzi*, PEM R22015), east bank of the Zambezi River opposite Ngonye Falls.

An individual (SVL 56.3 mm) was photographed (Fig. 3c) at Ngonye Falls campsite on 21 April 2013, while a second was photographed at the same site on 13 February 2014. This species has been recorded only once before in Zambia, in the extreme south at Kalamba Station on the old Zambezi Sawmill Railway (Broadley and Daele 2003). These new records are only the second and third confirmed records of this species in Zambia. This species was previously placed in the genus *Colopus*, but was transferred to *Pachydactylus* by Heinicke et al. (2017).

Hemidactylus mabouia (Moreau De Jonnès, 1818)

Common Tropical House Gecko

Material: PEM R22019, 22020; TM 86251, 86252

Photographs: VMUS 158888–158890, 158892

Seen on the walls of the visitor's center, Park Headquarters, and Ngonye Falls campsite. This species is common and widespread throughout Zambia (Broadley 1971a).

Lygodactylus chobiensis FitzSimons, 1932

Chobe Dwarf Gecko

Material: PEM R22026; TM 86253

Photographs: VMUS 158901, 158903–158906

The most common gecko species, seen on trees and walls in the vicinity of Ngonye Falls campsite, visitor's center, and Park Headquarters. Largely restricted to the Zambezi Valley (Branch 1998; Broadley 1971a), but should be searched for along the banks of the Cuando River.

Gerrhosauridae

Gerrhosaurus auritus Boettger, 1887

Kalahari Plated Lizard

Photograph: VMUS 158885

Individuals were photographed at Ngonye Falls visitor's center and Park Headquarters. We refer our individuals from Ngonye Falls to *G. auritus* on the basis of non-mucronate dorsal scales, the proximal caudal scales lacking spines, the large tympanum covering a large portion of the ear opening, and coloration. The only previous Zambian specimens are from Lealui, 170 km NNW, although it is also recorded 130 km to the south in the Zambezi Region of Namibia (Broadley 1971a).

Gerrhosaurus intermedius Lönnberg, 1907

Eastern Black-lined Plated Lizard

Photograph: VMUS 158887

A number of individuals suspected to be referable to *G. intermedius* were seen, however only a single individual was photographed and the scalation details later determined from this photograph (Fig. 2f). A narrow, pale dorsolateral line is present, bordered on each side by a dark line. A pale vertebral stripe, flanked on either side by a dark stripe, originates on the nape and extends to the base of the tail as discrete, evenly-spaced dashes. The frontonasal is divided into a large, heart-shaped anterior portion and a smaller posterior "inter-frontonasal," which is in contact with the frontal thus separating the prefrontals. The dorsal scales are arranged in 22 longitudinal and approximately 56 transverse rows. The flank scales are keeled and weakly mucronate and the head length is contained in the body length approximately 4.14 times.

Despite the prefrontals being separated, this individual is distinguished from *G. flavigularis* by the presence of four supraciliaries, the keeled and weakly mucronate lateral scales, head length into snout-vent length ratio and coloration (Bates et al. 2013; Branch 1998; FitzSimons 1943). It is distinguished from *G. auritus* by the number of longitudinal and transverse scale rows and the keeled and weakly mucronate lateral scales. We refer this individual to *G. intermedius* rather than *G. nigrolineatus sensu stricto* based predominantly on coloration. In *G. nigrolineatus* the dark stripes bordering the pale dorsolateral stripes are often ragged, while the pale vertebral stripe (and flanking dark stripes) may be continuous or absent, but are usually better-developed than in *G. intermedius* (Bates et al. 2013). When present, the dark

markings surrounding the discontinuous vertebral stripe appear to be more extensive (Bates et al. 2013; Hallowell 1857). This record is about 110 km NNW of the nearest previous reported locality at Sesheke (Broadley 1971a).

Lacertidae

Ichnotropis capensis (Smith, 1838)

Cape Rough-scaled Lizard

Material: PEM R22021–22024

Photographs: VMUS 158896, 158902

Individuals were recorded in the vicinity of Park Headquarters, Ngonye Falls campsite, visitor's center, eastern bank of the Zambezi River opposite Ngonye Falls, and also in Sioma Ngwezi National Park. It is widespread in western Zambia (Broadley 1971a).

Ichnotropis grandiceps Broadley, 1967

Zambezi Rough-scaled Lizard

Material: TM 86237

A number of individuals were encountered in Baikiaea woodland in the vicinity of Park Headquarters (Fig. 2d). All observed individuals were judged to be adult based on their size, and were seen in April and May. TM 86237 has a snout-vent length of 49.6 mm and a tail length of 101.8 mm. The frontonasal is entire, the occipital does not project beyond the parietals, and there are five supralabials anterior to the subocular, which borders the lip. The dorsal scales are strongly keeled and overlapping, and arranged in 47 rows at midbody. In all these characters our specimen closely resembles the type description by Broadley (1967). Furthermore, there are 14 femoral pores present on each thigh. In coloration, the specimen and photographed individuals closely resemble the holotype (USNM 163989, available: <http://inaturalist.ca/taxa/35953-Ichnotropis-grandiceps>; Accessed 5 December 2017). All individuals are similar in coloration with the head and back pale grey-brown to olive-brown anteriorly, becoming olive-yellow posteriorly, usually with scattered darker spots throughout. The limbs are reddish-orange, with small pale spots sometimes present on the hind limbs. A rust-red lateral stripe originates just behind the eye and extends for the entire length of the body, fading on the base of the tail. Below the lateral stripe the flanks are light grey, frequently with large white spots. The tail is uniformly grey-brown to pinkish, with a series of paired dorsolateral dark and white spots. The ventrum is white. This is the first record of this Data Deficient species in Zambia (Turner 2010), and these are the first specimens reported since Haacke (1970).

Meroles squamulosus (Peters, 1854)

Common Rough-scaled Sand Lizard

Photograph: VMUS 158907

An individual was photographed in a dry marsh in open woodland between Ngonye Falls campsite and visitor's center, while a second individual was photographed at the visitor's center. This species was also observed in Sioma Ngwezi National Park. These are the first records of this species in Western Province (Broadley 1971a), although it is known from northern Namibia (Branch 1998) and was recently recorded from south-eastern Angola (Conradie et al. 2016).

Scincidae

Acontias jappi (Broadley, 1968)

Barotse Legless Skink

Material: TM 86232–34, TM 86254

Individuals were collected in soil under bush clumps near Ngonye Falls campsite, airstrip and Park Headquarters (Fig. 2e). Numerous additional individuals were unearthed during the construction of Park Headquarters. Originally described as a subspecies of *Acontias kgalagadi* (previously *Typhlosaurus lineatus* [Boulenger, 1887]), Schneider and Bauer (2009) elevated this taxon to species status on morphological grounds. Specimens were distinguished from *A. k. kgalagadi* on the basis of being significantly more robust, having only two longitudinal dark stripes which fade out on the tail (typically 4–8 in *A. k. kgalagadi*); 24–25 subcaudal scales (26–35 in *A. k. kgalagadi*), the ocular scale being longer than high (as long as high in *A. k. kgalagadi*), and only three sublingual scales border the mental (usually four in *A. k. kgalagadi*; Broadley 1968b; Branch 1998). TM 86232–34 have four supralabials and three infralabials on each side, while TM 86233 has the mental longitudinally divided into two subequal parts (undivided in the remaining specimens). The superior border of the ocular scale is incompletely fused with the anterior supraciliary on both sides in all four specimens, resulting in a narrow slit that is apparently continuous with the eye (i.e., an immovable lower eyelid). Most other members of *Acontias* have the eye either completely covered by an ocular scale or have a moveable lower eyelid (Branch 1998), with only *A. rieppelli* having an immovable lower eyelid (Branch 1998). *Acontias jappi* is restricted to south-western Zambia and adjacent Angola (Broadley 1968b, 1971a; Schneider and Bauer 2009).

Acontias schmitzi Wagner, Broadley and Bauer, 2012

Schmitz's Legless Skink

Material: PEM R22015

A single specimen was collected on the east bank of the Zambezi River, opposite Ngonye Falls (Fig. 3d). It was found under a large log in deep sand in Miombo (*Brachystegia* spp.) woodland. Head scalation conforms to the type specimen. The new specimen measures 138 mm

snout-vent length and 21 mm tail length, has 14 mid-body scale rows, 178 ventrals and 26 subcaudals. Color in life is light orange ventrally and blue-grey dorsally, with the anterior two-thirds of the tail darkly pigmented ventrally. This is only the second record of this species and the most southerly locality. The holotype was collected in the Kataba Reserve, south of Mongu, Western Province, Zambia (15°23'00.9"S, 23°23'43.7"E; Wagner et al. 2012a), and this record is 140 km south of the type locality. Both records are on the eastern side of the Zambezi River in deep Kalahari sands. Based on morphological features, Wagner et al. (2012a) place *A. schmitzi* in a clade with *A. jappi*. Considering their distribution, it is expected that these two species are most probably sister taxa, and that the Zambezi River acts as a barrier to dispersal, thus facilitating their independent evolution and parapatric distribution. This species is endemic to Zambia.

Mochlus sundevallii (Smith, 1849)

Sundevall's Writhing Skink

Material: PEM R22027

Photograph: VMUS 158908

This semi-fossorial species was found at Ngonye Falls visitor's center as well as on the east bank of the Zambezi River opposite Ngonye Falls. It is widespread in Zambia (Broadley 1971a).

Panaspis maculicollis Jacobsen and Broadley, 2000

Spotted-neck Snake-eyed Skink

Photograph: VMUS 158911

Photographed in the vicinity of Ngonye Falls visitor's center. *A. Panaspis* seen on the east bank of the Zambezi River opposite Ngonye Falls is provisionally also assigned to this species. These records are situated between the previous records at Sesheke and Ndanda (Broadley 1971a; Jacobsen and Broadley 2000).

Typhlacontias rohani Angel, 1923

Kalahari Burrowing Skink

Material: TM 86235–36, 86248

Three specimens were collected during the construction of Park Headquarters, while additional individuals were photographed at the same locality as well as at Ngonye Falls campsite and the vicinity of the airstrip. All three specimens have five supralabials on either side of the head, with the second contacting the eye. On the right side of TM 86235, the prefrontal is separated from the frontoparietal by the enlarged third supraocular, while the prefrontal and frontoparietal are in contact on the left side of TM 86235 and on both sides of TM 86236. In Zambia this species was previously recorded only at

Kalabo in Western Province, where it is sympatric with *T. gracilis*, although it is widespread in north-western Zimbabwe, northern Botswana and Namibia, and south-eastern Angola (Branch 1998; Broadley 2000a; Conradie et al. 2016; Haacke 1997; Uetz et al. 2017).

Trachylepis damarana (Peters, 1870)

Damara Variable Skink

Material: PEM R22030, 22031

Photographs: VMUS 158929, 158930, 158933–158935

The most common skink in the area, seen at Ngonye Falls campsite, vicinity of the visitor's center, Park Headquarters, vicinity of the airstrip, and on the east bank of the Zambezi River opposite Ngonye Falls. It is common throughout Zambia (Broadley 1971a). Recently split from the larger *Trachylepis varia* complex by Weinell and Bauer (2018).

Trachylepis wahlbergii (Peters, 1869)

Wahlberg's Striped Skink

Material: PEM R22029

Photographs: VMUS 158931, 158936

Common throughout the area, seen at Ngonye Falls campsite, visitor's center, Park Headquarters, and on the east bank of the Zambezi River opposite Ngonye Falls. This species is common and widespread throughout Zambia, extending into Botswana and Namibia (Branch 1998; Broadley 1971a). Castiglia et al. (2006), using karyotypic and genetic data, suggest that *T. wahlbergii* is conspecific with *T. striata*, despite the morphological differences which prompted Broadley (2000b) to treat them as separate species.

Serpentes

Colubridae

Telescopus semiannulatus semiannulatus Smith, 1849

Eastern Tiger Snake

Photograph: VMUS 158924

Recorded on a number of occasions in the vicinity of Ngonye Falls campsite and visitor's center. This species appears to be widespread throughout Zambia (Broadley 1971a).

Dispholidus typus viridis (Smith, 1828)

Green Boomslang

Photographs: VMUS 158882, 158884

Regularly recorded at Ngonye Falls campsite, around the visitor's center, as well as at Idjobwa Pan in the northern buffer zone of Sioma Ngwezi National Park. Widespread in the southern provinces of Zambia (Broadley 1971a).

Thelotornis capensis oatesii (Günther, 1881)

Oates's Vine Snake

Photograph: VMUS 158925, 158926, 158928

Commonly encountered at Ngonye Falls campsite, and widespread in Zambia (Broadley 1971a).

Philothamnus angolensis Bocage, 1882

Western Green Snake

Material: PEM R22028; TM 86231

Photograph: VMUS 158913

A common, diurnal species seen regularly along the banks of the Zambezi River. One individual (VMUS 158913) was photographed at Ngonye Falls campsite, one (TM 86231) had drowned in the Zambezi River and one (PEM R22028) was collected on the eastern bank of the Zambezi River below Ngonye Falls. It is widespread in Western Province, as well as in the northern provinces of Zambia (Broadley 1971a).

Natricidae

Limnophis bangweolicus (Mertens, 1936)

Eastern Striped Swamp Snake

Material: PEM R22926; TM 86203, 86249

One individual (TM 86203) was found emerging from the Zambezi River at dusk at Ngonye Falls campsite; a second individual (TM 86249) was found dead and partially desiccated at the same site; while a third was found partially consumed on the east bank of the Zambezi River just below Ngonye Falls. Near-endemic.

Elapidae

Dendroaspis polylepis Günther, 1864

Black Mamba

Photographs: VMUS 158880, 158881, 158883

Regularly observed at Ngonye Falls campsite and visitor's center. The species is widespread in Zambia (Broadley 1971a).

Naja nigricollis Reinhardt, 1843

Black-necked Spitting Cobra

Photographs: VMUS 158909, 158910

Individuals were encountered regularly at Ngonye Falls campsite and near the visitor's center. This species occurs widely in central and northern Zambia, but is largely replaced by *N. mossambica* in the south (Broadley 1971a; Broadley et al. 2003).

Lamprophiidae

Amblyodipsas polylepis (Bocage, 1873)

Common Purple-glossed Snake

Photograph: VMUS 158870

Individuals were photographed at Ngonye Falls campsite and Park Headquarters. This species is widespread in Zambia, and is particularly common in sandy regions (Branch 1998; Broadley 1971a,c; Broadley et al. 2003).

Hemirhagerrhis nototaenia (Günther, 1864)

Eastern Bark Snake

Photographs: VMUS 158891, 158893–158895, 158900

Observed frequently at Ngonye Falls campsite, around the visitor's center, and at Park Headquarters. The species is widespread in Zambia (Broadley 1971a).

Lycophidion multimaculatum Boettger, 1888

Spotted Wolf Snake

Photograph: VMUS 158898

An individual was photographed at Ngonye Falls campsite. The species is widely distributed in western and northern Zambia (Broadley 1971a; Broadley et al. 2003).

Psammophis mossambicus Peters, 1882

Olive Sand Snake

Photographs: VMUS 158914–158916

Commonly encountered at Ngonye Falls campsite, visitor's center, Park Headquarters and on the roads in the vicinity. The species is common and widespread throughout Zambia (Broadley 1971a, 2002).

Psammophis subtaeniatus Peters, 1882

Western Stripe-bellied Sand Snake

Photograph: VMUS 158917

This diurnal species was often encountered at Ngonye Falls campsite, around the visitor's center, at Park Headquarters, Maziba Bay, and vicinity. Individuals were identified by coloration (including presence of a broad yellow mid-ventral band flanked on each side by a dark longitudinal stripe) and having the preocular in contact with the frontal (well-separated in *P. mossambicus*). This species has not previously been recorded from Western Province (Broadley 1971a, 2002; Broadley et al. 2003), having been recorded peripherally in Southern and Central Provinces, although more widespread in Eastern Province (Broadley 1971a, 2002). It is widespread in adjacent northern Namibia (Branch 1998; Broadley 2002). These records extend the known distribution by about 110 km to the north-west.

Pseudaspis cana (Linnaeus, 1758)

Mole Snake

Photographs: VMUS 158918, 158919, 158922, 158923

This common, diurnal snake was regularly seen in the vicinity of Ngonye Falls campsite, visitor's center, Park Headquarters, and surrounding area. A number of individuals were also killed by passing traffic on the tarred road running parallel to the Zambezi River. It is widespread in Zambia (Broadley 1971a).

Xenocalamus mechowii Peters, 1881

Elongate Quill-snouted Snake

Material: TM 86247

Photograph: VMUS 158937

Individuals were recorded at Ngonye Falls campsite and Park Headquarters, where a number of individuals (e.g., TM 86247) were unearthed during construction. Two subspecies have been historically recognized, which were separated on distribution and ventral and subcaudal scale counts (Peters 1881, Witte and Laurent 1947; Broadley 1971c). Broadley (1971c) notes a population of apparent intergrades in northern Zambia and this, together with the overlap in the supposedly diagnostic characters, lead us to not recognize these subspecies until a thorough review of these taxa has been undertaken.

Leptotyphlopidae

Leptotyphlops scutifrons (Peters, 1854)

Peters' Thread Snake

Material: PEM R22025

A single individual was collected in a pitfall trap on the east bank of the Zambezi River, opposite Ngonye Falls. The only previous Zambian locality is Kalichero in Eastern Province, although it has been recorded in Namibia at Katima Mulilo (Broadley and Broadley 1999).

Pythonidae

Python natalensis Smith, 1840

Southern African Python

Photograph: VMUS 158927

A number of individuals were seen around Ngonye Falls visitor's center and campsite, and locals also report the presence of this species in the vicinity. This species is widespread throughout Zambia (Broadley 1971a).

Typhlopidae

Afrotyphlops mucruso (Peters, 1854)

Zambezi Beaked Blind Snake

Material: TM 81409

Although not recorded by ourselves, this species has been collected at Ngonye Falls (TM 81409; Broadley and

Wallach 2009). This species is widespread in Zambia, while its sister species *A. schlegelii* has been collected south of the Zambezi River at Katima Mulilo (Broadley and Wallach 2009).

Afrotyphlops schmidtii (Laurent, 1956)

Schmidt's Beaked Blind Snake

Material: PEM R22016; TM 86246

Photograph: VMUS 158865

A juvenile specimen (PEM R22016) was collected in a pitfall trap on the eastern bank of the Zambezi River just above Ngonye Falls, while another specimen (TM 86246) was found dead on the dirt road leading to Sioma Barge. Individuals were regularly encountered around Ngonye Falls campsite and visitor's center, especially after rains. The nearest published locality is Kalabo (Broadley and Wallach 2009), about 210 km to the NNW.

Viperidae

Bitis arietans arietans Merrem, 1820

Puff Adder

Photographs: VMUS 158871, 158872, 158874

This species was often encountered at Ngonye Falls campsite and visitor's center, and is widespread in southern and central Zambia (Broadley 1971a).

Crocodylia

Crocodylidae

Crocodylus niloticus Laurenti, 1768

Nile Crocodile

Photographs: VMUS 158875, 158878, 158879

Observed in the Zambezi River both above and below Ngonye Falls, including at Maziba Bay and at Sioma Barge. It is widespread in Zambia and adjacent regions (Broadley 1971a).

Testudines

Testudinidae

Kinixys spekii Gray, 1863

Speke's Hinged Tortoise

Photograph: VMUS 158897

Seen around Park Headquarters and visitor's center. This species is widespread in Zambia (Broadley 1971a). These records are about 100 km north of the nearest known population in the Zambezi Region of Namibia (Branch 1998).

Stigmochelys pardalis (Bell, 1828)

Leopard Tortoise

Photograph: VMUS 158920

Seen in the vicinity of Park Headquarters, while a fresh carapace was confiscated from a poacher in Sioma Ngwezi National Park. Additional carapaces are on display in the visitor's center, and these may have been collected in the general vicinity of the study area. This species is most common in the Eastern Province of Zambia, but has also been recorded from Livingstone (Broadley 1971a).

Discussion

We report on three new reptile country records and one potentially new amphibian country record for Zambia, bringing the known herpetofauna to 86 amphibian, two crocodile, 10 chelonian, 78 lizard, and 91 snake species.

The herpetofauna of Ngonye Falls and surroundings is similar to that of south-eastern Angola (Conradie et al. 2016), with 13 amphibian and 34 reptile species in common. There are an additional 10 reptile and one amphibian species with closely related species in Angola. This is not surprising considering the similarity in habitat. However, the list of species in common is largely devoid of habitat specialists, suggesting that this apparent connectivity may not apply to all taxa. Although this may be an artifact of the relatively small sample sizes to date, these results also suggest that there are barriers to some species, most likely posed by the drainage basins. This was observed for some species (e.g., *Acontias* spp.: this study; *Elapsoidea* spp.: Broadley 1971b), for which the Zambezi River apparently acts as a dispersal barrier. Fossorial taxa are also largely absent in central Zambia (Wagner et al. 2012a), largely as a result of the Kalahari sands reaching their eastern limit not far beyond the Zambezi River. Vegetation, geology and natural barriers therefore all appear to play a role in shaping the herpetofauna of western Zambia.

Based on our present knowledge it would appear that members of the genus *Acontias* have parapatric distributions, with *A. k. kgalagadi* occurring west of the Cuando River and south of the Kavango River, *A. jappi* occurring between the Cuando and Zambezi Rivers, and *A. schmitzi* occurring east of the Zambezi River. The Zambezi River is likely to pose a formidable barrier to subterranean species such as *Acontias* and it is thus likely that this river effectively separates *A. jappi* and *A. schmitzi*. Furthermore, considering their distribution, it is probable that these two taxa are sister species (see also Wagner et al. 2012a). It will be informative to construct a dated phylogeny of these taxa to investigate whether the Zambezi River may have played a role in their speciation. The factors separating the distributions of *A. jappi* and *A. k. kgalagadi* are less clear. Although *A. k. kgalagadi* has thus far been recorded only west of the Cuando River, and *A. jappi* only to the east, the Kalahari sands extend beyond the source of the Cuando River, and this river may there-

fore only pose a local barrier between these two species. Additional sampling is required to more accurately determine the distribution of these two fossorial taxa, to investigate the probable barriers between them, and to determine whether they do in fact occur sympatrically anywhere.

Broadley (1967) described *Ichnotropis grandiceps* from three specimens collected 40 km west of Mohembo, Botswana, near the Botswana-Capriivi Strip (now the Zambezi Region of Namibia) border (ca. 18°19'03"S, 21°12'03"E). Haacke (1970) subsequently collected this species at Ndobe on the Namibia-Botswana border (ca. 19°34'41"S, 20°59'58"E, TM 30822), the farm Deo Volente near Grootfontein, Namibia (ca. 19°02'01"S, 18°46'29"E, TM 38309 and 38310) and on the Capriivi Strip-Botswana border 16 km east of the 21° corner beacon (i.e., approximately at the type locality). To the best of our knowledge, this species has not been seen or collected since Haacke (1970). In our experience *I. grandiceps* was a relatively common and active, diurnal species and it seems intriguing that it has not been recorded for more than four decades. The type specimens were collected in open woodland on Kalahari sands (Broadley 1967), while Haacke (1970) collected one specimen on hard limy soil in Combretum-Acacia bushveld, and three specimens on white sand in open bushveld. We found individuals in open to relatively closed Baikiaea woodland on pale, deep Kalahari sands. The type series consists of two adult males and a "juvenile," but measurements are only provided for the largest individual (Broadley 1967). Based on measurements presented in the article, three of the specimens collected by Haacke (1970) were adults, while the fourth individual was a subadult. Interestingly, all of the specimens collected to date have been taken in either April or May (Broadley 1967; Haacke 1970; this study). These are the first published records of this species in Zambia, with all previous records from south-west of the Kavango River. The presence of this species between the Cuando and Zambezi Rivers suggests that it may also occur in adjacent south-eastern Angola. However, to date, the only *Ichnotropis* species collected in south-eastern Angola do not match the description of *I. grandiceps* and appear to represent undescribed taxa (Conradie et al. 2016).

Our results suggest that western Zambia, and the region around Ngonye Falls in particular, has a suite of taxa in common with adjoining regions of Zambia and south-eastern Angola, as well as a suite of apparently unique taxa. However, most surveys to date have been restricted to the regions immediately adjacent to the Zambezi River, probably because of easy access, and it would be insightful to conduct surveys away from the Zambezi River to gain a better understanding of the entire herpetological assemblage in this region.

Our list should be regarded as preliminary, as numerous additional species are known from the general vicinity and are likely to be recorded here in the future. There

are unconfirmed sightings of *Acanthocercus atricollis atricollis* (Smith, 1849) from the east bank of the Zambezi River opposite Ngonye Falls, while we also observed an *Aparallactus capensis capensis* Smith, 1849 in Ngonye Falls campsite and *Crotaphopeltis hotamboeia* (Laurenti, 1768) individuals in the northern buffer zone of Sioma Ngwezi National Park at Idjobwa Pan, as well as in Ngonye Falls campsite, but did not secure photographic evidence or voucher specimens. *Varanus niloticus* (Linnaeus, 1766) was commonly observed in the Zambezi River in the vicinity of Ngonye Falls, while three juvenile lacertids with a striped dorsal pattern which were seen at Ngonye Falls campsite may be referable to *Nucras ornata* (Gray, 1864), but this remains to be verified. There are carapaces of *Pelomedusa subrufa* (Bonnaterre, 1789) and *Pelusios bechuanicus* FitzSimons, 1932 on display in the visitor's center, and although these are suspected to have been sourced from the vicinity of Ngonye Falls and/or Sioma Ngwezi National Park, this could not be verified.

Based on their occurrence in similar habitat in nearby areas, the following additional amphibian species are expected to occur in the region: *Leptopelis bocagii* (Günther, 1865); *Sclerophrys lemairii* (Boulenger, 1901); *Hyperolius nasutus* Günther, 1865; *Kassina senegalensis* (Duméril and Bibron, 1841); *Phrynomantis affinis* Boulenger, 1901; *P. bifasciatus* (Smith, 1847); *Hildebrandtia ornata* (Peters, 1876); *Phrynobatrachus mababienensis* FitzSimons, 1932; *Ptychadena grandisonae* Laurent, 1954; *P. nilotica* (Seetzen, 1855); *P. porosissima* (Steindachner, 1867); *P. taenioscelis* Laurent, 1954; *Pyxicephalus adspersus* Tschudi, 1838; and *Amnirana darlingi* (Boulenger, 1902) (Channing 2001; Conradie et al. 2016; Furman et al. 2015; Poynton and Broadley 1985a,b, 1987, 1988, 1991).

A number of additional reptile species are also expected to occur in the region based on their presence at nearby locations, including: *Pelusios rhodesianus* Hewitt, 1927; *Lygodactylus angolensis* Bocage, 1896; *Pachydactylus punctatus* Peters, 1854; *Varanus albigularis* Daudin, 1802; *Monopeltis mauricei* Parker, 1935; *Amblyodipsas ventrimaculata* (Roux, 1907), *Boaedon capensis* Duméril, Bibron and Duméril, 1854; *Crotaphopeltis barotseensis* Broadley, 1968; *Philothamnus semivariatus* (Smith, 1840); *Psammophis lineatus* (Duméril, Bibron and Duméril, 1854); *P. angolensis* (Bocage, 1872); *Dasyplectis scabra* (Linnaeus, 1758), *Naja anchietae* Bocage, 1879; *Atractaspis bibronii* Smith, 1849 and *Causus rhombeatus* (Lichtenstein, 1823) (Bayless 2002; Branch 1998; Broadley 1968, 1971a,c, 1991b, 1995, 2000a, 2002, 2014; Broadley et al. 1976; Conradie et al. 2016; Haagner et al. 2000; Hughes 2004; Loveridge 1958; Rasmussen 1997, 2005; Roux 1907).

Acknowledgements.—We are grateful to John Davies for field assistance, and to the Zambia Wildlife Authority (ZAWA) for granting us permission to search for rep-

tiles. WC thanks Michiel Jonker for field assistance and logistics. Roger Bills (South African Aquatic Biodiversity Institute) is thanked for providing additional material collected from the Ngonye Falls region. We thank William R. Branch, Michael F. Bates, and Philipp Wagner for commenting on, and greatly improving, an earlier draft of this manuscript.

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Appendix 1. List of amphibian and reptile species recorded at Ngonye Falls and surrounding regions in south-western Zambia, indicating voucher type and accession number. Museum acronyms are: PEM: Port Elizabeth Museum; SAIAB: South African Aquatic Biodiversity Institute, Grahamstown and TM: Ditsong National Museum of Natural History, Pretoria. All photographs are accessioned into the FrogMAP and ReptileMAP platforms of the Virtual Museum, Animal Demography Unit, University of Cape Town (available: vmus.adu.org.za).

Species	Voucher Number	Virtual Museum Number
ORDER: ANURA		FrogMAP
BREVICEPTIDAE		
<i>Breviceps adpersus adpersus</i> Peters, 1882		5982
<i>Breviceps poweri</i> Parker, 1934		5983
BUFONIDAE		
<i>Poyntonophrynus fenoulheti</i> (Hewitt and Methuen, 1913)		5989
<i>Schismaderma carens</i> (Smith, 1848)	SAIAB 205361, 205631	5992–93
<i>Sclerophrys gutturalis</i> (Power, 1927)		5994
<i>Sclerophrys poweri</i> (Hewitt, 1935)	SAIAB 205356	5996–97
<i>Sclerophrys pusilla</i> (Hallowell, 1855)	PEM A11719–20; SAIAB 205360	5995, 5998
HEMISOTIDAE		
<i>Hemisus marmoratus</i> (Peters, 1854)		5985
HYPEROLIIDAE		
<i>Hyperolius angolensis</i> Steindachner, 1867		5986
PHRYNOBATRACHIDAE		
<i>Phrynobatrachus natalensis</i> (Smith, 1849)	SAIAB 205351	5987
<i>Phrynobatrachus parvulus</i> (Boulenger, 1905)		5988
PIPIDAE		
<i>Xenopus muelleri</i> (Peters, 1844)	SAIAB 202357	
<i>Xenopus poweri</i> Hewitt, 1927	SAIAB 202355	
PTYCHADENIDAE		
<i>Ptychadena</i> cf. <i>mapacha</i> Channing, 1993	TM 86255	5990
<i>Ptychadena oxyrhynchus</i> (Smith, 1849)	SAIAB 205353–54	5991
<i>Ptychadena subpunctata</i> (Bocage, 1866)	PEM A11717–18; SAIAB 205358, 205365	
PYXICEPHALIDAE		
<i>Tomopterna</i> cf. <i>cryptotis</i> (Boulenger, 1907)	SAIAB 205362	5999, 6000
RHACOPHORIDAE		
<i>Chiromantis xerampelina</i> Peters, 1854		5984
ORDER: SQUAMATA		ReptileMAP
SAURIA – AGAMIDAE		
<i>Agama armata</i> Peters, 1855	PEM R22017–18	158866–69
AMPHISBAENIDAE		
<i>Dalophia longicauda</i> (Werner, 1915)		163523
<i>Dalophia pistillum</i> (Boettger, 1895)	PEM R22925	
<i>Monopeltis anchietae</i> (Bocage, 1873)	TM 86250	
<i>Zygaspis nigra</i> Broadley and Gans, 1969	TM 86209	158938
<i>Zygaspis quadrifrons</i> (Peters, 1862)	TM 86208	158939
CHAMAELEONIDAE		
<i>Chamaeleo dilepis</i> Leach, 1819		158873, 158876–77

Herpetofauna of Ngonye Falls

Appendix 1 (continued). List of amphibian and reptile species recorded at Ngonye Falls and surrounding regions in south-western Zambia, indicating voucher type and accession number. Museum acronyms are: PEM: Port Elizabeth Museum; SAIAB: South African Aquatic Biodiversity Institute, Grahamstown and TM: Ditsong National Museum of Natural History, Pretoria. All photographs are accessioned into the FrogMAP and ReptileMAP platforms of the Virtual Museum, Animal Demography Unit, University of Cape Town (available: vmus.adu.org.za).

Species	Voucher Number	Virtual Museum Number
GEKKONIDAE		
<i>Pachydactylus wahlbergii wahlbergii</i> (Peters, 1869)		163521–22
<i>Hemidactylus mabouia</i> (Moreau De Jonnès, 1818)	PEM R22019–20; TM 86251–52	158888–90, 158892
<i>Lygodactylus chobiensis</i> FitzSimons, 1932	PEM R22026; TM 86253	158901, 158903–06
GERRHOSAURIDAE		
<i>Gerrhosaurus auritus</i> Boettger, 1887		158885
<i>Gerrhosaurus intermedius</i> Lönnberg, 1907		158887
LACERTIDAE		
<i>Ichnotropis capensis</i> (Smith, 1838)	PEM R22021–24	158896, 158902
<i>Ichnotropis grandiceps</i> Broadley, 1967	TM 86237	
<i>Meroles squamulosus</i> (Peters, 1854)		158907
SCINCIDAE		
<i>Acontias jappi</i> (Broadley, 1968)	TM 86232–34, TM 86254	
<i>Acontias schmitzi</i> Wagner, Broadley and Bauer, 2012	PEM R22015	
<i>Mochlus sundevallii</i> (Smith, 1849)	PEM R22027	158908
<i>Panaspis maculicollis</i> Jacobsen and Broadley, 2000		158911
<i>Typhlacontias rohani</i> Angel, 1923	TM 86235–36, 86248	
<i>Trachylepis damarana</i> (Peters, 1870)	PEM R22030–31	158929–30, 158933–35
<i>Trachylepis wahlbergii</i> (Peters, 1869)	PEM R22029	158931, 158936
SERPENTES – COLUBRIDAE		
<i>Telescopus semiannulatus semiannulatus</i> Smith, 1849		158924
<i>Dispholidus typus viridis</i> (Smith, 1828)		158882, 158884
<i>Thelotornis capensis oatesii</i> (Günther, 1881)		158925–26, 158928
<i>Philothamnus angolensis</i> Bocage, 1882	PEM R22028; TM 86231	158913
NATRICIDAE		
<i>Limnophis bangweolicus</i> (Mertens, 1936)	TM 86203, 86249; PEM R22926	
ELAPIDAE		
<i>Dendroaspis polylepis</i> Günther, 1864		158880–81, 158883
<i>Naja nigricollis</i> Reinhardt, 1843		158909–10
LAMPROPHIIDAE		
<i>Amblyodipsas polylepis</i> (Bocage, 1873)		158870
<i>Hemirhagerhis nototaenia</i> (Günther, 1864)		158891, 158893–95, 158900
<i>Lycophidion multimaculatum</i> Boettger, 1888		158898
<i>Psammophis mossambicus</i> Peters, 1882		158914–16
<i>Psammophis subtaeniatus</i> Peters, 1882		158917
<i>Pseudaspis cana</i> (Linnaeus, 1758)		158918–19, 158922–23
<i>Xenocalamus mechowii</i> Peters, 1881	TM 86247	158937
LEPTOTYPHLOPIDAE		
<i>Leptotyphlops scutifrons</i> (Peters, 1854)	PEM R22025	

Appendix 1 (continued). List of amphibian and reptile species recorded at Ngonye Falls and surrounding regions in south-western Zambia, indicating voucher type and accession number. Museum acronyms are: PEM: Port Elizabeth Museum; SAIAB: South African Aquatic Biodiversity Institute, Grahamstown and TM: Ditsong National Museum of Natural History, Pretoria. All photographs are accessioned into the FrogMAP and ReptileMAP platforms of the Virtual Museum, Animal Demography Unit, University of Cape Town (available: vmus.adu.org.za).

Species	Voucher Number	Virtual Museum Number
PYTHONIDAE		
<i>Python natalensis</i> Smith, 1840		158927
TYPHLOPIDAE		
<i>Afrotyphlops mucruso</i> (Peters, 1854)	TM 81409	
<i>Afrotyphlops schmidtii</i> (Laurent, 1956)	PEM R22016; TM 86246	158865
VIPERIDAE		
<i>Bitis arietans arietans</i> Merrem, 1820		158871–72, 158874
ORDER: CROCODYLIA		
CROCODYLIDAE		
<i>Crocodylus niloticus</i> Laurenti, 1768		158875, 158878–79
ORDER: TESTUDINES		
TESTUDINIDAE		
<i>Kinixys spekii</i> Gray, 1863		158897
<i>Stigmochelys pardalis</i> (Bell, 1828)		158920



Darren Pietersen is a Ph.D. candidate at the University of Pretoria Department of Zoology and Entomology, and a research associate of the Ditsong National Museum of Natural History in Pretoria. His main interests are reptile taxonomy, as well as general reptile and amphibian surveys and the ecology of these taxa. Darren has authored or co-authored a number of scientific and popular articles. He has conducted herpetological surveys in a number of African countries, including Mozambique and the Democratic Republic of the Congo.



Errol Pietersen is a conservationist with a passion for reptiles and amphibians. His work has taken him to various interesting locations in Africa, where he has contributed to the knowledge of the herpetofauna of these regions. Errol has co-authored various popular articles on reptiles and amphibians, in particular on the diversity of these taxa at various sites in Mozambique.



Werner Conradie has ten years of experience in southern African herpetofauna, with his main research interests focusing on taxonomy, conservation, and ecology of amphibians and reptiles. He has published numerous principal and collaborative scientific papers and has served on a number of conservation and scientific panels, including the Reptile Atlas Committee and Amphibian IUCN Workshop. Werner has represented his field on television and in numerous field guides and has participated in expeditions in various countries including Namibia, Botswana, Zimbabwe, Mozambique, Angola, Malawi, Lesotho, and Zambia. He is currently the Curator of Herpetology at the Port Elizabeth Museum (Bayworld), South Africa.



Cryptic multicolored lizards in the *Polychrus marmoratus* Group (Squamata: Sauria: Polychrotidae) and the status of *Leiolepis auduboni* Hallowell

^{1,5}John C. Murphy, ²Richard M. Lehtinen, ³Stevland P. Charles,
^{1,4}Danielle Wasserman, ¹Tom Anton, ²Patrick J. Brennan

¹Science and Education, Field Museum of Natural History, 1400 Lake Shore Drive, Chicago, Illinois 60616 USA ²The College of Wooster, Department of Biology, 931 College Mall, Wooster, Ohio, 44691 USA ³Department of Biology, Howard University, 415 College Street NW, Washington, DC 20001 USA ⁴Vertebrate Museum, Department of Biology, Southeastern Louisiana University, Hammond, Louisiana 70402 USA

Abstract.—The Neotropical genus *Polychrus* contains seven species of arboreal lizards. The type species for the genus is the widespread *Polychrus marmoratus*. We compared a few populations of *P. marmoratus* using 16S and COI mitochondrial gene sequences (1,035 bp total) and found several lineages existing under the name *Polychrus marmoratus*. Working backwards, using morphology we identify *Polychrus marmoratus* from the Guiana Shield and resurrect the name *Leiolepis auduboni* Hallowell for the species present in Trinidad, Tobago, and northern Venezuela. The number of species in the genus *Polychrus* is raised to eight. However, we also discuss evidence for the existence of other cryptic species within *P. marmoratus*, and the likelihood that both *P. virescens* Schniz and *P. neovidanus* Wagler are valid names.

Keywords. Guyana Shield, Venezuelan Coastal Ranges, Trinidad, Tobago, Atlantic Forest, reptiles

Citation: Murphy JC, Lehtinen RM, Charles SP, Wasserman D, Anton T, Brennan PJ. 2017. Cryptic multicolored lizards in the *Polychrus marmoratus* Group (Squamata: Sauria: Polychrotidae) and the status of *Leiolepis auduboni* Hallowell. *Amphibian & Reptile Conservation* 11(1) [General Section]: 1–16 (e132).

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Received: 26 March 2016; **Accepted:** 04 September 2016; **Published:** 16 January 2017

Introduction

The multi-colored lizards of the genus *Polychrus* have traditionally been treated as part of the Iguanidae since Gray (1845) organized lizards into 24 families. Townsend et al. (2011) note that, while *Polychrus* and *Anolis* have been considered sister taxa (Frost and Etheridge 1989), all published analyses of molecular data contradict this finding (Frost et al. 2001; Schulte et al. 2003; Schulte and Cartwright 2009). Frost et al. (2001) defined Polychrotidae based on morphology-only in a combined molecular and morphological analysis. Using conventional alignment methods and a much larger molecular data set Schulte et al. (2003) failed to recover the Polychrotidae clade. More recently, Pyron et al. (2013) suggested *Polychrus* is more closely related to hoplocerids. Hoploceridae contains four genera and about 24 species of medium

sized Neotropical endemic saurians with spiny tails, pleurodont teeth, diurnal activity, arboreal life styles, and an omnivorous diet. Members of the genus *Hoplocercus* are dry forests lizards, while *Enyalioides* and *Morunasaurus* inhabit rain forests. *Polychrus*, on the other hand, spans a range of habitats, has an exceptionally long, non-spiny tail, and is diurnal, omnivorous and arboreal. The slow moving, arboreal *Polychrus* range from Honduras southward into continental South America on both sides of the Andes (Avila-Pires 1995; this study), extending as far as 25.63°S on the east side of the Andes.

Polychrus is composed of seven recognized species: *P. marmoratus* Linnaeus 1758, *P. acutirostris* Spix 1825, *P. gutturosus* Berthold 1846, *P. liogaster* Boulenger 1908, *P. femoralis* Werner 1910, *P. peruvianus* Noble 1924, and the most recently described *P. jacquelinae* Koch et al. 2011. Boulenger's (1914) *P. spurrelli* was considered a

Correspondence. Email: ⁵serpentresearch@gmail.com (Corresponding author)

subspecies of *P. gutturosus* by Peters and Donoso-Barros (1970), however Koch et al. (2011) considers the name a junior synonym of *P. gutturosus*.

Much of the distribution of *Polychrus* is occupied by the type species for the genus, *Polychrus marmoratus*. It occurs in South America east of the Andes, including Guyana, French Guyana, Suriname, Venezuela, Colombia, Ecuador, Peru, Brazil, and Paraguay (Hoogmoed 1973; Avila-Pires 1995). Vanzolini (1983) noted *P. marmoratus*' distribution was disjunct with a large gap in northeastern Brazil separating the Caribbean and northern Amazonian populations from the Atlantic Forest populations. A museum specimen (MCZ R-25135) documents Breder's (1946) report of its presence in southern Panama. Other museum specimens suggest its presence in Valparaíso, Chile (MCZ R-3369) and elsewhere on the west side of the Andes. The species also occurs on the Isla de Margarita (Ugueto and Rivas 2010), Trinidad (Court 1858), Tobago (Barbour, 1916), and on the Bocas Islands of Gaspar Grande, Monos, and Chacachacare (Boos 1984a, b, 1990). This distribution covers about 37° of latitude and 45° of longitude with populations ranging in elevation from sea level to at least 2,500 m above sea level (asl) in Venezuela. Rivas et al. (2012) reported *Polychrus marmoratus* occurs in all of the biogeographic divisions of Venezuela except the marine, coastal, and Pantepui divisions. Molina et al. (2004) suggests it is present in the gallery forests and swamp forests of the llanos. It is not likely to be present in open grasslands. Figure 1 illustrates specimens from six different portions of the range.

Lacerta marmorata Linnaeus (1758:208) was based upon specimens documented in the Museum Principis and the Amphibia Gyllenborgiana. Specimens from the later collection were missing when Lönnberg (1896) examined the collections. However, Holm (1957) later found four specimens in the Amphibia Gyllenborgiana apparently overlooked by Lönnberg (1896). Cuvier (1817) established the genus *Polychrus* for *Lacerta marmorata*, citing Lacépède's (1788) illustration which he considered accurate enough for identification. Hoogmoed (1973) restricted the type locality for *Lacerta marmorata* to Paramaribo, Suriname. A specimen collected by Wied-Neuwied at the Villa Viçosa, Bahia, Brazil was the basis for the description of *Polychrus virescens* Schniz (1822). However, when Wied-Neuwied (1822–1831) published on this specimen, he referred to it as *P. marmoratus*.

Fitzinger (1826) listed the name *Polychrus geometricus* as *patria ignota* (locality unknown) and Vanzolini (1983) considered it *nomen nudum*. Delaporte (1826) described *Polychrus fasciatus* based upon a mounted specimen in the Muséum National d'Histoire Naturelle (Paris) and reported its type locality as the Philippines or Moluccas. This specimen was not discussed by Duméril and Bibron (1837) nor Guibé (1954) and remains *nomen dubia*.

Wagler (1828) described *Polychrus strigiventris* in a single sentence in the same account he discusses *Poly-*

chrus virescens. He states that the femoral pores and coloration distinguish it from the other species but, provides no type locality. Wagler's *Polychrus strigiventris* is also a *nomen dubia*.

Wagler (1833a) described *Polychrus neovidanus* (based on Seba's (1734–1765) plate 76, figure 4 and a Spix specimen from Rio de Janeiro) on the basis that the specimen lacked obvious femoral pores (it was a female). Vanzolini (1983) proposed the name *P. neovidanus* Wagler which should be associated with Spix's specimen from Rio de Janeiro.

Hallowell (1845) described *Leiolepis auduboni* collected by Samuel Ashmead at a location within 200 miles of Caracas, Venezuela. Hallowell's specimen was identified as *Polychrus marmoratus* by Roze (1958), however its status as a junior synonym of *Polychrus marmoratus* has been overlooked in more recent works (Peters and Donoso-Barros 1970; Avila-Pires 1995).

Numerous taxonomic changes due to underestimated diversity in other Neotropical lizards (Giugliano et al. 2013; Domingos et al. 2014; Werneck et al. 2015) suggests *Polychrus marmoratus* may also be a good candidate for holding undescribed, cryptic species. *Polychrus marmoratus* has not been examined in detail since Hoogmoed (1973) and Avila-Pires (1995) provided species accounts in their catalogues for Suriname and Brazil. Here, we focus on northeastern South America, define *Polychrus marmoratus*, and discuss possible cryptic species within the species.

Methods and Materials

We examined 118 alcohol preserved specimens and 17 skeletal and cleared and stained museum specimens labeled *Polychrus marmoratus*, as well as five specimens labeled *Polychrus liogaster* (listed in species accounts, Appendix 1 lists other material examined). Localities with precise information were plotted using ArcView. We also used localities from VertNet and the literature to provide an overall view of the distribution of the *Polychrus marmoratus* group. Preserved specimens were fixed in formalin and stored in 70% ethanol. Morphological data was collected (JCM, SPC, DW, TA) and morphological nomenclature used follows Hoogmoed (1973) and Avila-Pires (1995). Body and tail lengths were taken to the nearest one mm with a metric ruler, and head and scale measurements were taken with dial and digital calipers. Values for paired head scales are given in left/right order. Univariate analyses of morphological data were conducted with Excel in combination with QI Macros. Abbreviations used include: n: number of specimens, X: mean value, SD: standard deviation, SVL: snout vent length, and asl: above sea level.

Two tissue samples were collected by RML in Tobago while the remaining tissue samples were obtained via loan from various museums (see Table 1 for list of localities). Total genomic DNA was extracted from tis-



Fig. 1. A–B: *Polychrus auduboni* from the Arima Valley, Trinidad (JCM). C–D: *Polychrus marmoratus*, probably from Suriname, (Twan Leenders). E: *Polychrus* sp. from 4.5 km S of Cumanacoa, Venezuela at 300–400 m asl (Walter E. Schargel). F: *Polychrus* sp. from Vitória do Xingu, Pará, Brazil (Pedro Peloso). G: *Polychrus* sp. Camamu, Bahia, Brazil. H: *Polychrus* sp. from Guarapari, Espírito Santo, Brazil (bottom) (Pedro Peloso).

sues using a DNeasy blood and tissue kit (Qiagen, Inc., Valencia, California, USA) following the manufacturer's instructions. Using a Qiagen TopTaq PCR Master Mix kit, we amplified a fragment of the cytochrome oxidase 1 (hereafter, COI) mitochondrial gene (~ 650 bp) using the primers LCO1490 and HCO2198 from Castañeda and de Queiroz (2011). We also amplified a ~ 430 bp fragment of the 16S mitochondrial gene using primers from Haas et al. (1993). Thermocycler conditions for PCR followed Castañeda and de Queiroz (2011).

Amplicons were purified using a Qiagen Min-Elute column purification kit and sequenced on an ABI PRISM 3100xl automated sequencer at the Molecular and Cellular Imaging Center at the Ohio Agricultural Research and Development Center, Ohio State University using the PCR primers. Bases were called in Codon Code Aligner (version 4.0.1). Sequences for each gene were aligned in MEGA 6.06 (Tamura et al. 2013) using the Clustal W

module with the default gap opening and gap extension penalties. Alignments were unambiguous.

We used both parsimony and maximum likelihood analyses in MEGA to analyze the combined aligned sequences of 16S and COI (1,035 bp total). For our maximum likelihood analysis, we used the Hasegawa-Kishino-Yano model of molecular evolution as it was supported by the Bayesian Information Criterion as the closest fit to our data using MEGA. Initial tree(s) for the heuristic search were obtained by applying Neighbor-Joining algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with the best log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories [+G, parameter = 0.1811]). One thousand bootstrap pseudoreplicates were used to assess topological support. In the parsimony analysis,

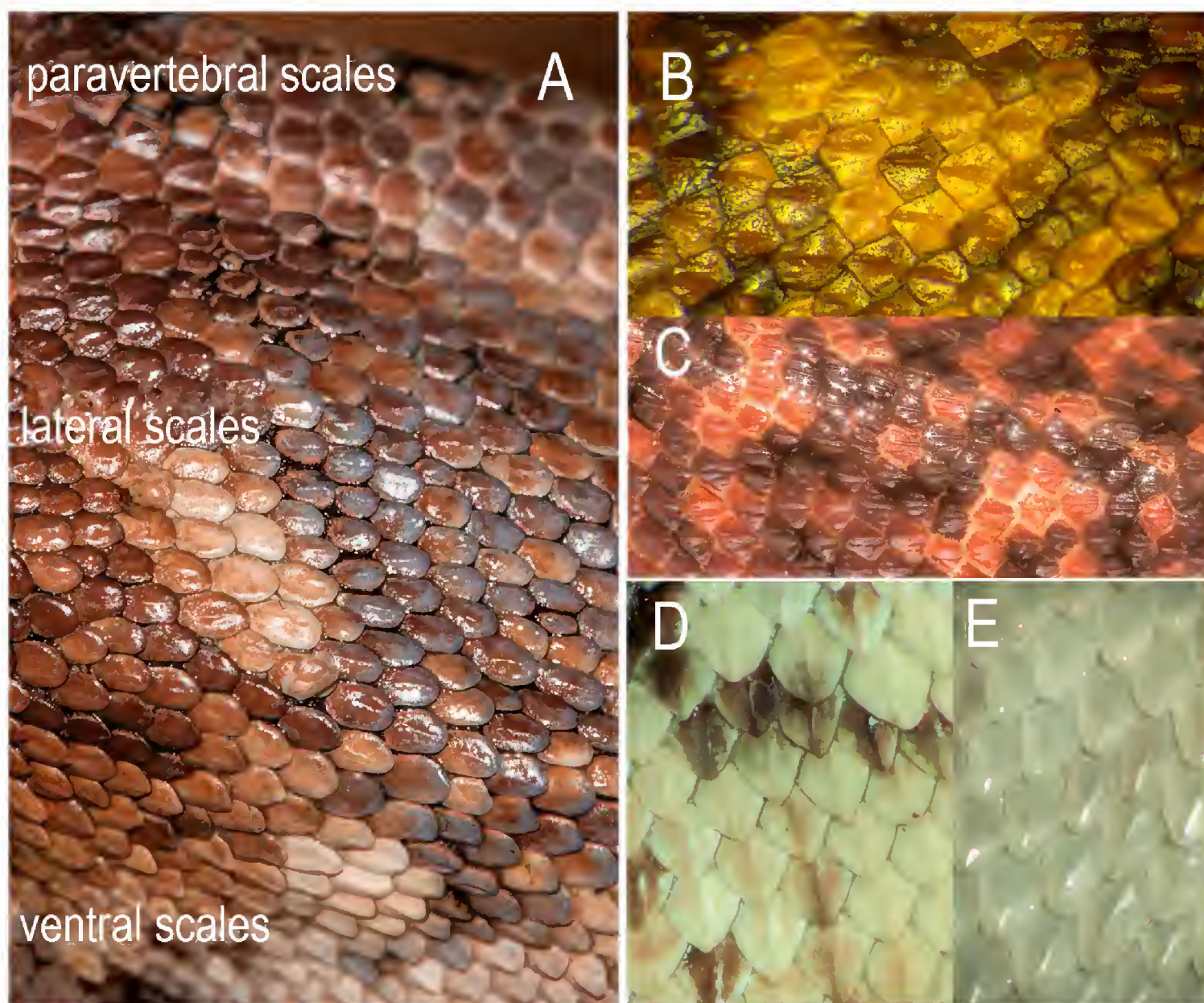


Fig. 2. **A:** the paravertebral, lateral and ventral scales of *Polychrus marmoratus*. **B:** paravertebral scales of *Polychrus auduboni*, a single keel per scale. **C:** paravertebral scales of *Polychrus marmoratus* with multiple keels per scale (multicarinate). **D:** ventral scales of *Polychrus auduboni* with keel extending the length of the scale. **E:** ventral scales of *Polychrus marmoratus* showing short keels, with some terminating in a small swollen gland at the apex of some scales (FMNH 3294, Demerara, Guyana).

all characters were unweighted and we used the subtree pruning-regrafting search method to identify the most parsimonious tree(s). Five thousand bootstrap pseudo-replicates were used to assess topological support. In all analyses, all codon positions were used, positions with less than 90% site coverage were eliminated, and sequences obtained from *P. acutirostris* were used as an outgroup (Table 1). Uncorrected p-distances were calculated for all pairs of sequences for each gene separately, using default settings in MEGA.

Collected specimens were covered by Trinidad and Tobago Forestry Division Special Game Licenses issued to JCM and RML on June 18, 2013 and June 5, 2014.

Results

The *Polychrus marmoratus* Group members share: 85–119 vertebral scales counted between the occiput to the posterior margin of the hind legs with a single keel or multiple keels; nasal scale contacts upper labials 1–2 or 2–3; first pair of chin shields may be in contact, or not; vertebral crest is absent; parietal eye absent; gular crest greatly reduced; 62–93 scales around mid-body; 23–35 lamellae on the fourth finger; 30–44 lamellae on the fourth toe; 10–18 semicircle scales; 5–8 rows of supra-oculars; 8–28 total pores; weak to strongly keeled ventral scales. Scales on the snout (those anterior to semicircle

scales) differ in ornamentation and organization within species, making it difficult to obtain consistent characters. The canthals (scales between the preorbitals and the supranasal) numbered two or three. The loreal is bordered by the canthals, the preorbitals, the upper labials, the nasal, and sometimes the suborbitals. The loreal can be single, divided into two parts, or fragmented into multiple parts and is variable within species. *Polychrus marmoratus* group members lack a gular crest but does have conical shaped scales on the mid line of the gular region. Paravertebrals (scales on the vertebral line and similarly shaped scales on either side of it) are in 8 to 14 transverse rows. These scales differ from lateral scales in ornamentation and size. They are often flat and may be ovate or polygonal with a single keel or two or three keels (multicarinate scales). The ornamentation of these is relatively consistent within species. Paravertebrals may be similar in size to lateral scales, or larger or smaller. Lateral scales are usually convex with a keel. They may be oval or quadrangular, and the interstitial skin contains numerous tiny granules. Ventral scales are triangular, larger than laterals, imbricate, and keeled. The keels may extend the entirety of the scale or only part of its length, and keels may end in a swollen bulb. The transition zone between laterals and ventrals make ventral counts ambiguous. See Figure 2 for the scale shapes and transition zones and other scale characters. *Polychrus marmoratus* has been

Cryptic multicolored lizards in the *Polychrus marmoratus* Group

Table 1. Specimens examined for molecular work, their geographic origins and Genbank accession numbers.

Specimen	Locality	COI	16S
YPM H-013040	none (pet trade specimen)	KY458391	KY458408
YPM H-014659	none (pet trade specimen)	KY458392	KY458409
KU 212631	Peru, San Martin, 14 km ESE of Shapaja	KY458396	KY458413
MVZ 163071	Peru, Amazonas, vicinity of Sua (Aguaruna village), Rio Cenepa (4°34'12.00"S, 78°13'18.01"W)	KY458406	KY458423
MVZ 230130	none (pet trade specimen, <i>P. acutirostris</i>)	KY458407	KY458424
LSUMZ 14270	Brazil, Para, Agropecuaria Treviso, LTDA, ca 101 km south, 18 km east Santarem (3°09'2.4"S, 54°50'32.9"W)	KY458398	KY458415
LSUMZ 14271	Brazil, Para, Agropecuaria Treviso, LTDA, ca 101 km south, 18 km east Santarem (3°09'2.4"S, 54°50'32.9"W)	KY458395	KY458412
LSUMZ 14392	Brazil, Para, Agropecuaria Treviso, LTDA, ca 101 km south, 18 km east Santarem (3°09'10.2"S, 54°50'28.4"W)	KY458397	KY458414
LSUMZ 4458	Trinidad and Tobago: Trinidad, San Fernando	KY458399	KY458416
AMNH 138080	Guyana: Northern Rupununi Savanna, Yupukari (on Rupununi River), 7 mi (airline) SSW Karanambo, 370 ft	KY458393	KY458410
AMNH 139787	Guyana: Southern Rupununi Savanna, Aishalton (on Kubanawau Creek), 150 m, (2°28'31"N 59°19'16"W)	KY458404	KY458421
CAS 231770	Trinidad and Tobago: Trinidad, Nariva Road, Manzanilla Beach. (10°29'25.6"N, 61° 03'16.8"W)	KY458402	KY458419
CAS 231781	Trinidad and Tobago: Trinidad, 5 km E of Laguna Mar Beach Resort, Blanchisseuse. (10°47'39.9"N, 61°17' 46.4" W)	KY458403	KY458420
UWIZM.2012.27.61	Trinidad and Tobago: Trinidad, Arima Valley, (10° 41' 5.57"N, 61°16'54.12"W)	KY458401	KY458418
UWIZM.2012.27.47	Trinidad and Tobago: Tobago, Arnos Valle Bridge Courtland River (~11°12'21.59"N, 60°45'35.99"W)	KY458400	KY458417
UWIZM.2012.42.12	Trinidad and Tobago: Tobago, 1.5 km upstream from Bloody Bay river bridge	KY458394	KY458411
RML Charlotteville	Trinidad and Tobago: Tobago, west side of Charlotteville (tissue collected, specimen discarded)	KY458405	KY458422

described as having three dark eye stripes: one extending posteriorly over the supratemporals, one extending to the rictus, and one extending ventrally to the upper labials. All specimens examined for this study have these markings, but in some specimens the pigment has faded from light and chemicals. Coloration in *Polychrus marmoratus* is highly variable and we have not emphasized coloration descriptions in alcohol or life for this reason.

The maximum likelihood tree of our 16S and COI sequence data provide evidence for a clade in Trinidad and Tobago but with little inter-island differentiation (Fig. 3). Both sequences from Guyana group together with high support, but are strongly divergent from all other sequences. There is also modest support for a grouping of the three Brazilian sequences, but the position of the Peruvian sequences is not well resolved. The parsimony analysis resulted in a highly similar tree with similar support (not shown).

The molecular phylogenetic results are supported by the p-distance data (Tables 2 and 3). There is little variation within sequences from Trinidad and Tobago (mean 16S p-distance 0.3% mean COI p-distance 0.1%) but

a moderate amount when compared to sequences from elsewhere (mean 16S p-distance 1.5% mean COI p-distance 1.6%). While the two Guyana sequences are nearly identical (16S p-distance 0.5%, COI p-distance 0.3%), they are well differentiated from all the other sequences (mean 16S p-distance 2.1% mean COI p-distance 3.1%). The three samples from Pará, Brazil are identical in their 16S sequence (mean 16S p-distance 0.0%) but somewhat different for COI (mean COI p-distance 1.1%). Compared to all the other sequences, the Brazil samples are moderately differentiated (mean 16S p-distance 1.1% mean COI p-distance 2.1%).

Polychrus head scale arrangements and counts are quite variable and working backwards from the molecular data we found a single species present on Trinidad and Tobago that morphologically appears conspecific with some Venezuelan Caribbean Coastal Range populations. Guyana and Suriname populations are molecularly and morphologically distinct from the Trinidad-Tobago-Venezuela species. The molecular results also suggest a third lineage is present in Para, Brazil.

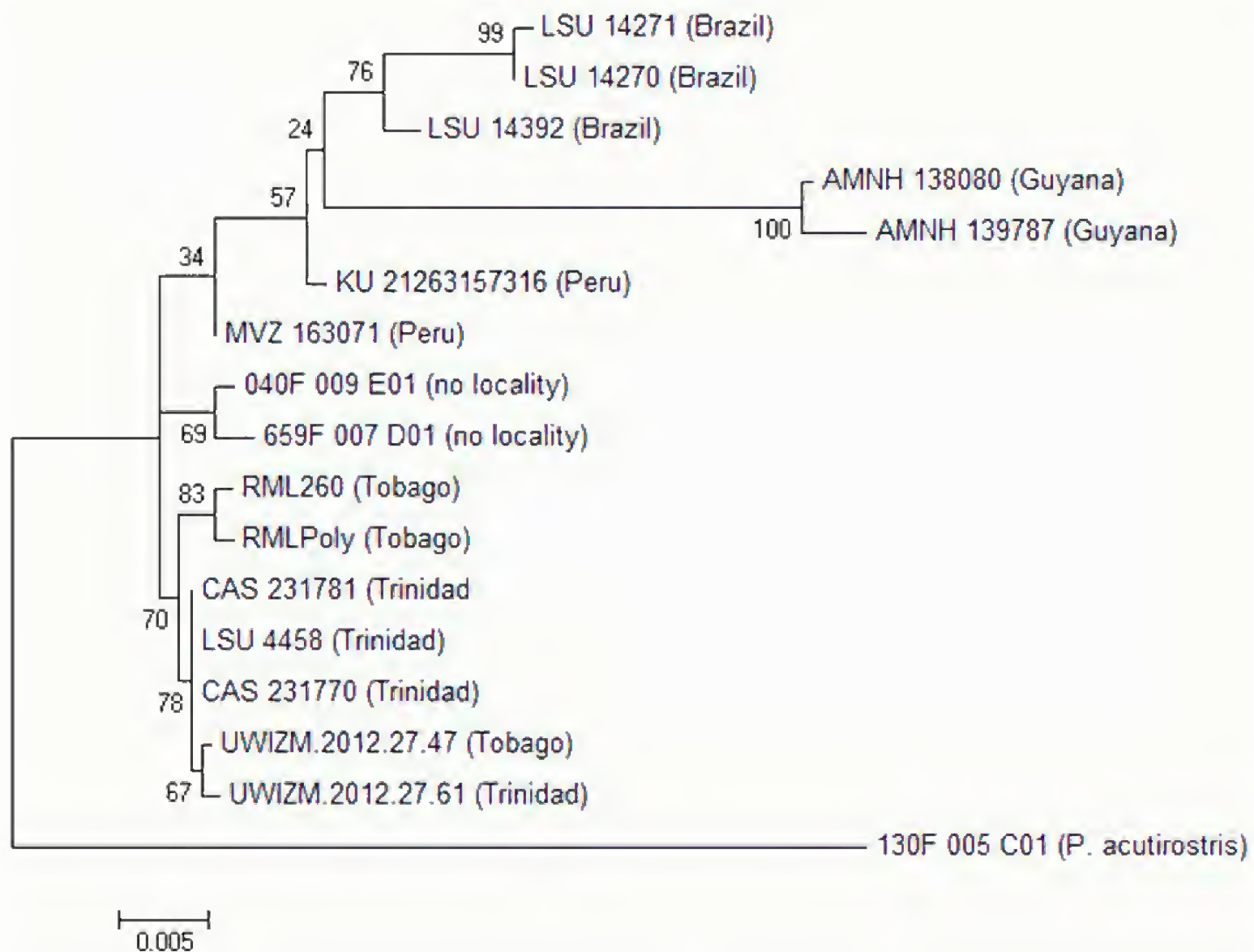


Fig. 3. Consensus maximum likelihood tree for combined 16S and COI sequence data from seventeen *Polychrus* tissue samples (1,035 bp total). Bootstrap support values are indicated at each node, if greater than 50%. Samples are indicated by their museum accession number and country of origin, if known. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. For details of analysis, see text.

Localities for the sources of *Polychrus* DNA, localities from which collected morphological data, type localities, and localities from which *P. marmoratus* has been reported are shown in Figure 4.

In a comparison of Trinidad ($n = 3$) and Guyana ($n = 1$) *Polychrus* skulls, one of us (DW) found the following. The parietal in Trinidad specimens had a slight constriction where the lateral borders give way to the posterior processes. The dorsal crests of the posterior processes angled medially so that, from the dorsal perspective, the lateral surfaces are visible and the medially surfaces are obscured. The lateral edges of the parietal table form slight bony lips that do not overhang the lateral surfaces of the parietal, which are supramedially sloped and un-depressed. Braincase: on the supraoccipital process, from the posterior perspective, there is a median seam running the length of the process, which extends to the rim of the foramen magnum (Fig. 5).

The parietal in Guyana specimens had the lateral edges of the parietal table project laterally, each forming a shelf that overhangs a depression in the adjacent lateral surface of the parietal bone. From the dorsal view, the shape of the parietal table is square and is un-constricted where the lateral borders give way to the posterior processes. The dorsal crests of the posterior processes angle laterally so that, from the dorsal perspective, the medial surfaces are visible and the lateral surfaces are obscured.

The braincase of Guyana specimens lacked a hairline median crest on the supraoccipital process.

The combined molecular and morphological data suggests *Polychrus marmoratus* is composed of multiple lineages. The morphology of the lineage present on Trinidad, Tobago, and the Coastal Ranges of Venezuela agrees well with the morphology of the holotype of *Leiolepis auduboni* Hallowell, including the morphology of the specimens we sequenced from Trinidad (UWIZM 2012.27.61) and Tobago (UWIZM 2012.27.47).

***Polychrus auduboni* (Hallowell) new combination**

Figure 6 (holotype), 1a, b

Leiolepis auduboni Hallowell 1845: ANSP 8138, Type locality: “Colombia within 200 miles of Caracas.” Collected by Samuel Ashmead. Restricted here to La Cumbre, Maracay, Aragua, Venezuela (~10.233333 -67.333336).

Polychrus marmoratus marmoratus – Burt and Burt 1933: 41. [in part]

Polychrus marmoratus – Roze 1958:2.

Referred specimens: Skeletal and cleared and stained specimens examined: Trinidad. AMNH 148543 female; FMNH 49840, FMNH 49845, FMNH 49848, UF 18395, males, UF 18922. Venezuela. FMNH

Table 2. Uncorrected p-distances estimating the evolutionary divergence between 16S sequences. The proportion of nucleotides that differ are shown, including all codons. All positions with less than 75% site coverage were eliminated. There were a total of 428 positions in the final dataset. Analyses were conducted in MEGA 6.06 (Tamura et al. 2013).

YPM 13040 (no locality)																
YPM 14659 (no locality)	0.002															
AMNH 138080 (Guyana)	0.019	0.021														
RML260 (Tobago)	0.009	0.012	0.023													
LSU 14271 (Brazil)	0.012	0.014	0.023	0.012												
KU 212631(Peru)	0.012	0.014	0.019	0.012	0.005											
LSU 14392 (Brazil)	0.012	0.014	0.023	0.012	0.000	0.005										
LSU 14270 (Brazil)	0.012	0.014	0.023	0.012	0.000	0.005	0.000									
LSU 4458 (Trinidad)	0.007	0.009	0.021	0.002	0.009	0.009	0.009	0.009								
UWIZM.2012.27.47 (Tobago)	0.009	0.012	0.023	0.005	0.012	0.012	0.012	0.012	0.012	0.002						
UWIZM.2012.27.61 (Trinidad)	0.009	0.012	0.023	0.005	0.012	0.012	0.012	0.012	0.012	0.002	0.002					
CAS 231770 (Trinidad)	0.007	0.009	0.021	0.002	0.009	0.009	0.009	0.009	0.009	0.000	0.002	0.002				
CAS 231781 (Trinidad)	0.007	0.009	0.021	0.002	0.009	0.009	0.009	0.009	0.009	0.000	0.002	0.002	0.000			
AMNH 139787 (Guyana)	0.019	0.021	0.005	0.023	0.026	0.021	0.026	0.026	0.021	0.023	0.023	0.021	0.021			
RML Charlotteville (Tobago)	0.009	0.012	0.023	0.005	0.012	0.012	0.012	0.012	0.002	0.005	0.005	0.002	0.002	0.023		
MVZ 163071 (Peru)	0.012	0.014	0.019	0.012	0.005	0.000	0.005	0.005	0.010	0.012	0.012	0.010	0.010	0.022	0.012	
MVZ 230130 (<i>P. acutirostris</i>)	0.098	0.100	0.103	0.098	0.100	0.100	0.100	0.100	0.095	0.098	0.098	0.095	0.095	0.100	0.095	0.101

17791 (Sucre, Venezuela) male. Alcohol specimens examined: *Polychrus auduboni* ($n = 66$). Tobago. UWIZM.2012.27.47, Arnos Valle Bridge Courtland River (~11.206 -60.760), UWIZM.2012.42.12, Bloody Bay River Bridge (11.301070 -60.626965); St. John; Charlotteville; Charles Turpin Estate (11.3165 -60.5499) FMNH 217257, USNM 227928–30, UWIZM 2011.30.2 UWIMZ CAREC.R.129, UWIMZ2012.27.42 Charlotteville, Tobago (11.3165 -60.5499). Trinidad. Arima Valley (10.684883 -61.281702) UWIZM 2012.27.61, Bush-bush (10.360255 -61.090106), Curepe (10.636930 -61.405493), UWIZM2010.12.49; Brickfield(10.455021 -61.467952) FMNH 49839, 49841, 49843, 49844, 49846; San Rafael (10.57174 -61.2642325) FMNH 49847, 49849; Port-Of-Spain (10.666667 -61.50579) MCZ R-79119–79123; San Fernando (10.2833 -61.4667) R-100484–87, R-119881; Tunapuna-Piarco (10.585543 -61.329526) CM S4846, S6520, S6534, S6539, S6543, S6561; Trinidad (no specific locality) MCZ R-12065, R-145299, R-145300–07, R-68888, R-69417, UMMZ 123692, UWIZM 2010.12.47a–c, BMNH 92.9.10.2, 97.7.23.17. Venezuela. Aragua (10.233333 -67.333336), CM S7412, S7425; UMMZ 124309; Distrito Capital (10.46786 -66.90625); CM 22797, 64748, MCZ R-109009, Falcon (11.016667 -68.566666), R-48729-30, R-49053; La Culebra, base of Duida (3.7299633 -65.80171967) R-58330; Monagas (10.2 -63.533) R-9981; Sucre (10.147126 -63.808614), MCZ R-50202, CM S7874, S7915, S7918, S7949; within 200 mi from Caracas ANSP 8138; Uroma, Yaracuay (10.48.3337 -68.31668) FMNH 29189–91.

Diagnosis: Loreal usually fragmented into two scales; supranasal not in contact with loreal; two or three internasals; loreal contacts upper labials 2–3–4; vertebral rows 93–112; scales around mid-body 62–80; lamellae

on fourth toe 30–43; usually three scales (2–4) between the first canthals, and five scales (4–6) between the second canthals; nasal does not usually contact first upper labial, but does contact the second and third; total femoral pores 17–28; multicarinate scales in paravertebral rows few or none; paravertebrals much larger than laterals; the number of supraocular rows usually five, rarely four or six; scales on anterior of snout finely striated with scattered tiny tubercles; scales on anterior surface of humerus keeled.

Re-description of holotype: Hallowell’s (1845) description for this species is of little or no use in distinguishing it from *Polychrus marmoratus* or other taxa in this genus. Based upon photographs and the original description: Adult female with a snout-vent-length (SVL) of 101 mm. The head is 0.29 of the SVL; 0.65 times longer than wide, as wide as high. Snout blunt. Neck narrower than the head, almost as wide as the anterior portion of the body. Body compressed. Tail almost round in cross section, tapering toward the tip, 2.83 times SVL. Rostral pentagonal, almost two times as wide as high, visible from above, bordered posteriorly by two large postrostral scales. Scales on snout heterogeneous in size, irregularly polygonal, juxtaposed, rugose. Three scales across snout between anterior canthals, five scales across snout between posterior canthals, two canthals between nasal and supraciliaries, anterior one largest. Supranasals separated by three scales across the snout. Supraorbital semicircles more or less distinct, with 6/7 scales, separated medially by one row of scales, slightly smaller in size than those of supraorbital semicircles. Thirty-eight scales in supraocular region distinctly smaller than those on snout, polygonal to rounded, juxtaposed, flat and smooth, irregularly arranged except a row of smaller scales adjacent to supraciliaries. Supraciliaries 8/9, juxtaposed, smooth, in a continuous series with canthals. Scales in

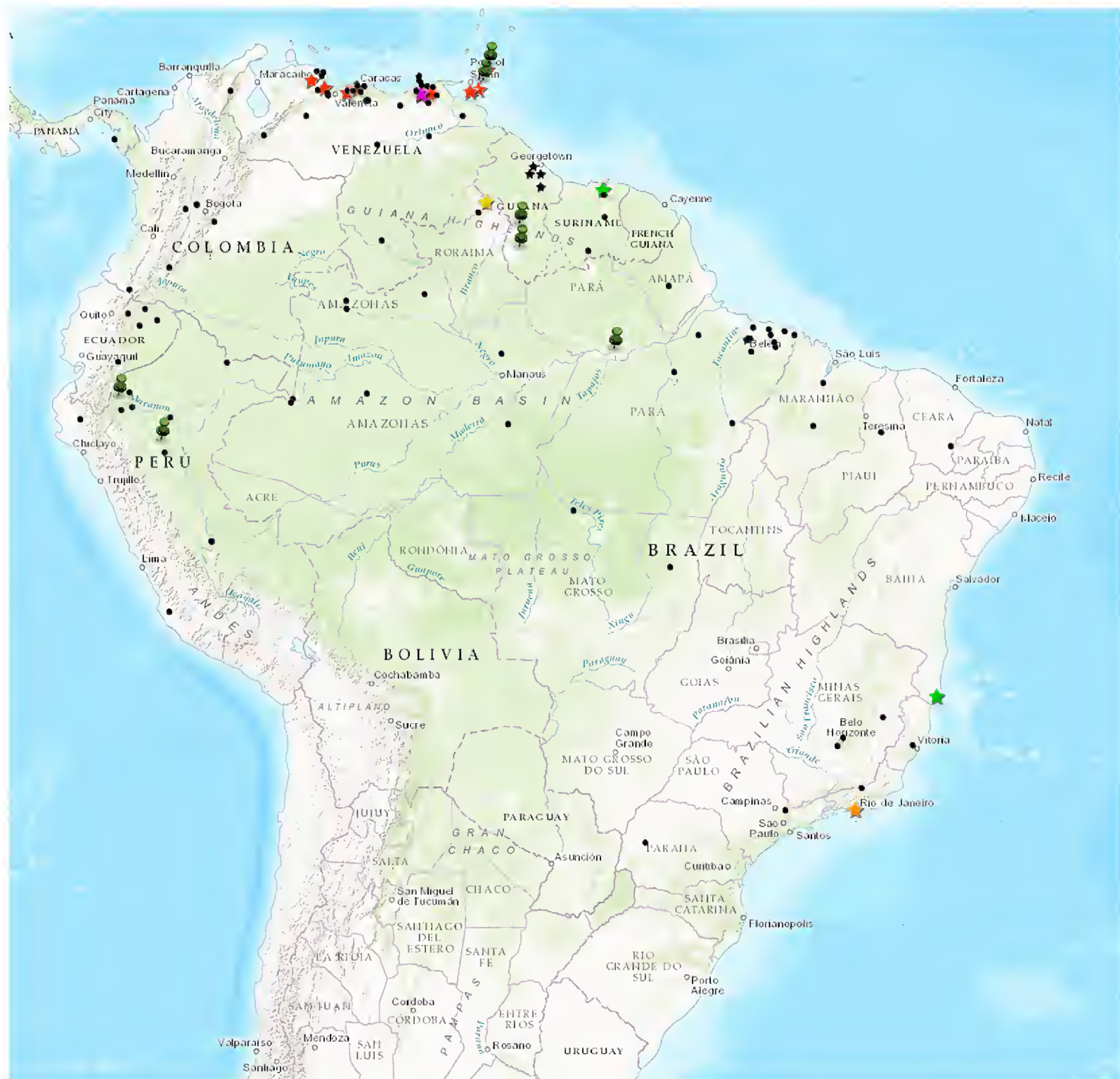


Fig. 4. Localities of members of the *Polychrus marmoratus* Group. Green pushpins denote localities from which DNA was sampled. Red stars denote locations for *Polychrus auduboni*, black stars denote locations for *P. marmoratus*. Black circular markers represent unconfirmed locations for members of the *P. marmoratus* group based upon the literature and specimens reported in VertNet. The blue star is the type locality for *P. marmoratus* (Linnaeus). The purple star is the type locality of *P. auduboni* Hallowell. The green star is the type locality for *Polychrus virescens* Schniz, the orange star is the type locality for *P. neovidanus* Wagler.

the parietal region are irregular polygonal, juxtaposed, flat, smooth, intermediate in size between those on snout and on supraocular region. Scales in interparietal region polygonal, juxtaposed, rugose, some somewhat swollen. Parietal eye absent. Loreal region with two scales. Nostril directed laterally, in upper anterior of a single nasal, nasal pentagonal and in contact with first and second supralabial. Orbit length 0.29 times head length. Eyelids partially fused, covered by granules of almost same size throughout the eyelids. A continuous series of two preoculars and two suboculars, in direct contact with supralabials and four postoculars. Upper labials seven on both sides, followed to rictus by a few relatively small scales. Temporal region with seven vertical rows of polygonal or rounded, juxtaposed, flat, and smooth scales, followed by two or three rows of granular scales near the ear, delimited dorsally by a single row of five or six enlarged supratemporal scales. Ear opening vertically oval with smooth margin, tympanum superficial. Mental bell-shaped, almost 2.5 times wide as long. Lower labials taller than

upper labials, six on each side, followed by several small scales to rictus. Lateral scales on chin slightly larger than median scales. Gular crest reduced but present, composed of about 10 enlarged, conical scales between mental and dewlap (anterior most obscured by thread holding tag). Dewlap has scales the same size and structure as chin scales, which are separated from each other by an extensible skin covered with granules, reaches level of forelimbs. Scales on anterior nape relatively small, granular, and almost rounded, juxtaposed, convex, in about 17 rows, posteriorly grading into dorsals. Scales on the sides of the neck about the same size as those on the nape but more elongated, merging ventrally with the gulars. Paravertebrals polygonal to rounded, juxtaposed, to some extent convex but mostly flat and keeled, 110 paravertebral scales in a mid-dorsal line between the occiput and the posterior margin of the hind limbs. Lateral scales are of a similar size and same shape as those of dorsum, convex, smooth, in poorly defined, oblique rows. Ventrals larger than dorsals, flat, keeled, lanceo-

Table 3. Uncorrected p-distances estimating the evolutionary divergence between COI sequences. The proportion of nucleotides that differ are shown, including all codons. All positions with less than 75% site coverage were eliminated. There were a total of 635 positions in the final dataset. Analyses were conducted in MEGA 6.06 (Tamura et al. 2013).

YPM 13040 (no locality)																	
YPM 14659 (no locality)	0.003																
AMNH 138080 (Guyana)	0.028	0.032															
RML260 (Tobago)	0.006	0.006	0.031														
LSU 14271 (Brazil)	0.020	0.021	0.033	0.020													
KU 212631 (Peru)	0.006	0.010	0.028	0.009	0.017												
LSU 14392 (Brazil)	0.008	0.011	0.031	0.011	0.016	0.008											
LSU 14270 (Brazil)	0.019	0.019	0.031	0.019	0.002	0.016	0.015										
LSU 4458 (Trinidad)	0.003	0.003	0.031	0.003	0.024	0.009	0.011	0.022									
UWIZM.2012.27.47 (Tobago)	0.003	0.003	0.031	0.003	0.024	0.009	0.011	0.022	0.000								
UWIZM.2012.27.61 (Trinidad)	0.003	0.003	0.031	0.003	0.024	0.009	0.011	0.022	0.000	0.000							
CAS 231770 (Trinidad)	0.003	0.003	0.031	0.003	0.024	0.009	0.011	0.022	0.000	0.000	0.000						
CAS 231781 (Trinidad)	0.003	0.003	0.031	0.003	0.024	0.009	0.011	0.022	0.000	0.000	0.000	0.000					
AMNH 139787 (Guyana)	0.031	0.036	0.003	0.035	0.036	0.031	0.034	0.035	0.035	0.035	0.035	0.035	0.035				
RML Charlotteville (Tobago)	0.006	0.006	0.031	0.000	0.020	0.009	0.011	0.019	0.003	0.003	0.003	0.003	0.003	0.035			
MVZ 163071 (Peru)	0.003	0.003	0.032	0.003	0.024	0.010	0.011	0.023	0.000	0.000	0.000	0.000	0.000	0.036	0.003		
MVZ 230130 (<i>P. acutirostris</i>)	0.000	0.002	0.031	0.004	0.020	0.004	0.007	0.018	0.002	0.002	0.002	0.002	0.002	0.035	0.004	0.002	

late, and imbricate, in poorly defined, oblique and transverse rows. Scales around mid-body about 80. Preanal plate has scales which are slightly smaller than ventrals, in nine rows. Preanal pores absent. Femoral pores indicated on 14/13 notched scales (female). Tail has rhomboidal, flat, sharply keeled scales, distinctly larger than the dorsals, arranged in longitudinal and oblique rows; keels aligned longitudinally; on ventral surface of the tail, scales are slightly larger and more rectangular. Tail not regenerated ending somewhat bluntly. Scales on forelimbs slightly larger than dorsals, polygonal to rounded, flat, keeled, mostly imbricate but more juxtaposed on upper arm, slightly smaller on ventral aspect of forearms, towards posterior aspect ventrals become smaller. Scales on hind limbs are as large as dorsals, polygonal to rounded, keeled, smooth, and imbricate on thigh and ventrally, juxtaposed on dorsal surface of tibia, slightly larger and slightly keeled on ventral part of tibia, and slightly lanceolate. Toward posterior aspect of thighs, both dorsally and ventrally, scales become distinctly smaller. Subdigital lamellae of fingers and toes single, short, with multiple keels, 25 under fourth finger, 34 under fourth toe.

Variation: We examined 66 specimens of this species. Females were significantly larger than males in size ($p < 0.05$, 53 df). Body length: females SVL $X = 116.3$ ($n = 31$), males SVL $X = 105.1$ ($n = 24$). Tail length: females $X = 291.9$ ($n = 26$), males $X = 277.1$ ($n = 19$), statistical tests inconclusive as to significant difference. Forelegs 0.30–0.48 of the SVL, $X = 0.37$, $SD = 0.51$; hind legs 0.37–0.65 of the SVL $X = 0.52$, $SD = 0.60$. Rostral broader than tall, contacts two postrostrals. Scales on snout slightly imbricate. Nasal with large nare, single supranasal above on the snout, separated by 1–3 internasals. Supranasal rarely makes contact with loreal. First pair of canthals (most anterior) separated by three (rarely two) scales, second pair of canthals separated by 4–6 scales

(usually 5). Semicircle scales 10–16, the total bilateral average 12.4, they were usually separated by a single scale (or scale row) anteriorly, and a double row posteriorly. Ciliaries 10–13, usually with two keels, supraocular scales polygonal to round in five or six rows between ciliaries and semicircle scales, first row or first two rows of supraoculars with keels (first row sometimes appears to be a second row of ciliaries), supraoculars highly variable in number (26–44). A prenasal scale usually separated the postrostral from the nasal. The nasal was mostly in contact with second upper labial, sometimes in narrow contact with the first or the third upper labial. Loreal usually in two parts, an anterior quadrangular scale and a posterior triangular scale; loreal usually contacts upper labials 2–3–4, sometimes 2–3 or 3–4; first canthal usually makes contact with loreal; loreal usually not in contact with supranasal. Upper labials 6–9 per side, usually seven and the bilateral average for total upper labials was 14.86; fifth upper labial usually under middle of eye, sometimes the seam of 4–5, and rarely the seam of 5–6. Lower labials 6–9, usually seven, and the total bilateral average for lower labials was 14.8 scales. Orbital scales: preorbitals two or three, rarely one; suborbitals usually two; and postorbitals usually three or four, rarely two. Temporal scales usually in 7–8 vertical rows between post orbitals and tympanum, separated from parietal scales by a row of 4–5 enlarged supratemporals. Gulars (counted between tympani) 39–60 ($X = 46.4$), rows of gulars separated by numerous micro-scales that vary in size. Dorsal scales in 62–80 rows around mid-body ($X = 71.5$); 93–112 scales along the vertebral line from the occiput to the posterior edge of the hind legs; on the vertebral line 10–14 rows of slightly enlarged, pentagonal to hexagonal, keeled scales, which gradually become elongate and ovate laterally, often losing the keels laterally (note these may appear as a mid-dorsal stripe) tran-

sitioning into sharply keeled, pointed, imbricate ventrals; scales similar to the ventrals in size and shape extend onto the anal plate. Scales on tail pentagonal, heavily keeled, and imbricate. Scales on limbs keeled, imbricate, and slightly more ovate proximally than distally.

Coloration: In alcohol the specimen is a uniform brown, see Fig. 5. In life, highly variable, may change quickly from bright green with white lateral blotches bordered in black to almost a uniform brown. See Figure 1a, b.

Distribution: *Polychrus auduboni* occurs in the Cordillera de Costa of Venezuela, Trinidad and Tobago. It is also likely present on the Isla de Margarita, in the forested areas of the llanos, and Orinoco Delta. However, it may be replaced by another species in Bolivar, Venezuela.

Natural History: Beebe (1944) discussed the natural history of this lizard. Unfortunately, he combines information from both Caripito Venezuela and Kartabo, Guyana and it is not possible to unravel the comments he makes about *P. marmoratus* from Guyana, and from this species from Venezuela. However, Test et al. (1966) observed *P. auduboni* in the Botanical Garden at Caracas and above Turiamo Pass, Aragua, Venezuela. The latter individual was kept alive and is described as moving slowly and deliberately, unless grasshoppers were placed in its cage, at which time it jumps or runs towards the insects. The authors also describe tail waving as a human approached the cage, and interpret this as defensive behavior. During the day, the lizard was green in color but changed to gray-green at night while it slept on a branch. Its prey may also include humming birds. We have observed this lizard on Trinidad and Tobago, active during the day in secondary forests, climbing through branches and drinking water from the surface of leaves. Several times we have observed pairs together in the same or adjacent trees. Known predators include the hawk *Leucopternis albicollis*, house cats, and the parrot snake *Lep-*

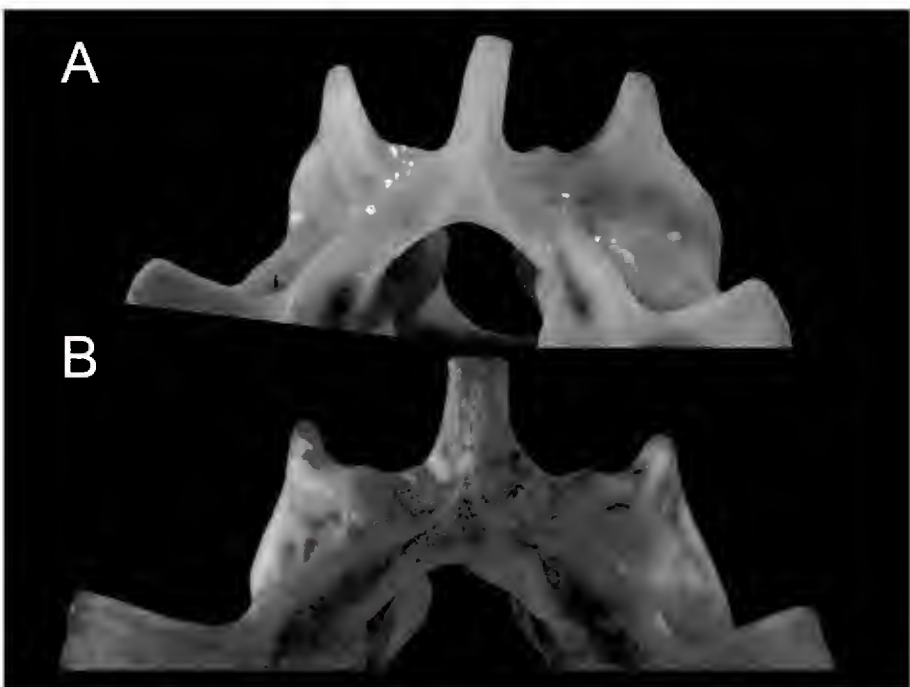


Fig. 5. On the supraoccipital process, from the posterior perspective, the presence of a median seam that runs the length of the process, down to the rim of the foramen magnum (DW).

tophis coeruleodorsus (Murphy 1997; Renoir Auguste, pers. comm.).

Comparisons: Distinguished from *Polychrus marmoratus* by few or no multicarinate paravertebral scales on the dorsum (many in *P. marmoratus*); paravertebrals consistently larger than laterals; loreal is one or two scales (3–4 in *P. marmoratus*); 17–28 femoral pores (8–19 in *P. marmoratus*).

***Polychrus marmoratus* (Linnaeus)**

Figures 1c, d.
Lacerta marmorata Linnaeus 1758: 208. Type locality “Hispania.” Restricted to the vicinity of Paramaribo, Suriname by Hoogmoed (1973).
Polychrus marmoratus – Cuvier 1817: 41.
Psilocercus marmoratus – Wagler 1821: 341.
Agama marmorata – Daudin 1802: 433.
Polychrus marmoratus marmoratus – Burt and Burt, 1933: 41.

Table 4. A comparison of *Polychrus marmoratus* and *P. auduboni* morphology.

Character	<i>P. auduboni</i>	<i>P. marmoratus</i>
<i>n</i> =	66	32
loreal scales (usual number)	1–2	2–3
vertebral rows, range	93–112100.34	95–113108.2
mean = SD =	(6.15)	(5.17)
scales around mid–body, range mean = SD =	62–8071.52 (6.15)	63–9378.5 (8.17)
nasal contacts upper labials	2 or 2–3	1–2
total semicircle scales	10–16	12–18
total pores, range mean = SD =	17–2821.41 (2.91)	8–1914.31 (3.31)
canthals	2	2 or 3
multicarinate paravertebrals	few or none	many
first labial contacts the nasal	rarely	usually



Fig. 6. ANSP 8138. The holotype for *Leiolepis auduboni* Hallowell. Photo credit: Ned Gilmore.

Referred specimens: Skeletal material: Suriname AMNH 141130, AMNH 148544 male, AMNH 141084, UF 56618 (Guyana or Suriname), UF 60914 female, UF 68102. Alcohol material: ($n = 32$). Guyana, Demerara, (6.733 -57.983) FMNH 3294, Dunoon Demerara River UMMZ 47632, 53965, 47630, 47751, 47752, 47753, 47754; Cabacalli Island Moruco River (6.78915 -58.182949) UMMZ 56467, 56468; Wismar (5.9999 -58.30001) UMMZ 76685; Kartabo (6.377459 -58.706761) UMMZ 47631, CM S4244, S4245, S5361; no specific locality; MCZ R-24391, UMMZ 47633, 55839, 55856; Suriname, Paramaribo (5.442523 -55.09896); MCZ R-8255, CM 44369, 52384, 52385, 44362–44368, 49531–32.

Diagnosis: Loreal usually fragmented into three or four scales; supranasal scale frequently (about 0.50) makes contact with loreal. One to three internasals. Vertebral rows 95–117; scales around mid-body 66–90. Snout length is 0.58 of orbit length. Lamellae on fourth toe 35–44. Usually three scales between first canthals, and five between the second; nasal contacts first two upper labials. Total femoral pores 8–19. Many multicarinate paravertebral scales; paravertebrals not much larger than laterals. The number of supraocular rows usually six (rarely 5 or 7). Scales on snout and supraoculars finely striated to smooth with tiny tubercles. Scales on anterior surface of humerus keeled; a reduced gular crest starts at the level of the first or second lower labial.

Variation: Females are significantly larger than males. Females SVL $X = 114.5$ ($n = 29$), males SVL $X = 102.9$ ($n = 11$) ($p = <0.05$, 29 df). Tail length: females $X = 292.3$ ($n = 16$), males $X = 272$ ($n = 10$). Forelegs $X =$

0.40 of SVL; $r = 0.35$ –0.47; hind legs $X = 0.52$ of SVL, $r = 0.45$ –0.57.

Rostral broader than tall, contacts two or three postrostrals. Scales on snout juxtaposed. Nasal with large nare, one or two supranasal above nasal on the snout, separated by 3–5 scales. Supranasal frequently makes contact with loreal. First pair of canthals (most anterior pair) separated by three (rarely two) scales; second pair of canthals separated by 4–5 scales (usually five). Semi-circle scales 5–9, the total bilateral average 14.4; usually separated by a single scale (or scale row) anteriorly, and a double row posteriorly. Ciliaries 10–13, usually with two keels. Supraocular scales polygonal to round in five or six rows between ciliaries and semicircle scales, first row or first two rows of supraoculars with keels (first row sometimes appears to be a second row of ciliaries); supraoculars highly variable in number (31–44) and in five or six rows (rarely seven). A prenasal scale usually separates the postrostral from the nasal. The nasal is usually square and in contact with the first two upper labials, sometimes in narrow contact with the third upper labial. Loreal usually in two or three parts, anterior scale quadrangular and posterior triangular scale. Loreal usually contacts upper labials 2–3–4, sometimes 2–3 or 3–4; first canthal usually makes contact with loreal. Upper labials numbered 5–9 per side, usually six; bilateral average for total upper labials 14.7; upper labial under middle of eye usually fifth, or 4–5, or 5–6. Lower labials 6–9, usually 7; total bilateral average for lower labials 14.1 scales. Orbital scales: preorbitals two; suborbitals 2–4, usually 2–3; postorbitals usually two, sometimes 3 or 4. Tem-

Table 5. A comparison of eight species currently recognized in the genus *Polychrus*. K = keeled, r = reduced, nd = no data, both means smooth and keeled in same species. Based on the literature for species not examined in this study.

	<i>acutirostris</i>	<i>auduboni</i>	<i>femoralis</i>	<i>gutturosus</i>	<i>jacquelinae</i>	<i>liogaster</i>	<i>marmoratus</i>	<i>peruvianus</i>
vertebrals	111–126	93–112	nd	75–105	198–215	103–125	95–113	56–70
SAB	57–73	62–80	53–73	63–82	131–186	66–95	63–93	52–74
ventral ornamentation	none	k	no	k	no	both	k	k
parietal eye	yes	no	no	no	no	no	no	no
vertebral crest	no	no	no	no	no	no	no	yes
gular crest obvious	no	r	no	no	no	yes	r	yes
4th finger	23–32	23–34	nd	25–36	33–36	29–37	23–35	25–33
4th toe	19–32	30–43	nd	35–45	42–48	38–47	34–44	32–43
femoral pores	23–24	17–28	30–34	18–42	13–15	15–24	8–19	12–26

poral scales usually in eight vertical rows between post orbitals and tympanum, separated from parietal scales by a row of 4–5 enlarged supratemporals. Gulars (counted between tympani 42–55 ($X = 47.0$)), rows of gulars separated by numerous micro-scales that vary in size. Dorsal scales around mid-body $X = 78.5$ ($r = 66–90$, $SD = 8.71$). Vertebrals and paravertebrals in 10–14 rows; slightly enlarged, pentagonal, and keeled; radially become elongate and ovate laterally often losing the keels; and transition into sharply keeled, pointed, imbricate ventrals. Scales similar to the ventrals in size and shape extend onto the anal plate. Vertebral scales rows $X = 108.2$ ($r = 95–113$, $SD = 5.17$) between the occiput and posterior edge of hind legs. Lamellae on fourth finger 25–35 ($X = 30.5$, $SD = 3.18$), lamellae on fourth toe 30–43 ($X = 38.69$, $SD = 3.01$). Total pores 8–19 ($X = 14.3$, $SD = 3.31$). Scales on tail pentagonal, heavily keeled, and imbricate. Scales on limbs keeled, imbricate, and slightly more ovate near the body, than distally.

Coloration: In preservative most specimens are a uniform brown-tan with black pigmented eye stripes and labial seams. In life, coloration is highly variable and can change in less than a minute from green to brown. See Figures 1c,d.

Distribution: We have only documented this species from Guyana and Suriname, but it may be expected to occur in French Guyana and northern Brazil.

Natural history: Hoogmoed (1973) described it as being diurnal, arboreal, and omnivorous; females lay 4–6 eggs in July and August. It is capable of rapid color change from bright green to brown. Comments on its natural history in the literature are deeply entangled with other members of the species group.

Comparisons: Distinguished from *Polychrus auduboni* by numerous multicarinate paravertebral scales (few or none in *P. auduboni*); paravertebrals not much larger than laterals; a loreal fragmented in two or more parts (as opposed to one or two in *P. auduboni*); a nasal that is pentagonal and usually contacts the first upper labial (it usually does not in *auduboni*); 8–19 ($X = 14.32$, $SD = 3.31$) femoral pores (*auduboni* has 17–28, $X = 21.4$, $SD = 2.91$); the supranasal often makes contact with the loreal (in *auduboni* it rarely does so).

Discussion

We have been unable to examine specimens from the west side of the Andes and Panama. However, we suspect the localities are in error, or the lizards have been misidentified. It is likely that members of the *Polychrus marmoratus* group are restricted to the east side of the Andes.

Given the high degree of morphological variation within the *P. marmoratus* complex, there are likely other cryptic species present. The *P. marmoratus* group will likely also include populations currently considered *Polychrus liogaster*. The two species are supposedly separated by keeled ventrals in *P. marmoratus* and smooth or weakly keeled scales in *P. liogaster* (Peters and Donoso-Barrio 1970). Additionally, the latter species has a post-ocular stripe that extends onto the neck and body. However, preliminary examination of museum specimens labeled *P. liogaster* revealed that all had keeled ventrals, as well as slightly higher upper labial counts than those reported in the literature.

We cannot rule out the possibility that other taxa remain to be found within *Polychrus auduboni* in fact, we think it likely. Ugueto and Rivas (2010) note that the Isla de Margarita population has a red dewlap and a white vertebral stripe. A mainland Venezuelan specimen (CM 22797) that we have tentatively included in *auduboni*, also has a red dewlap. Only recently have we observed a red dewlap in a Trinidad *Polychrus auduboni*. Additionally, we have observed *P. auduboni* often have a broad vertebral stripe that frequently contains white pigment. This may be normal variation in a species capable of significant changes in coloration, or it may signal a cryptic species within *P. auduboni*. Two specimens from Cocolar, Sucre (FMNH 17792–93) also appear to be distinct from *P. auduboni*, with the lowest number of vertebral scale counts (85) we observed in *Polychrus* and an exceptionally well defined canthal ridge. A single specimen from Bolivar, Venezuela (UMMZ 85232) does not appear to be *P. auduboni* or *P. marmoratus*. It has three postrostrals, exceptionally small nape scales that are tuberculate, and other traits that are uncommon or unobserved in our sample. Barrio-Amorós and Ortiz (2015)

suggest an undescribed *Polychrus* exists in eastern Venezuela. It is unclear if they are referring to *P. auduboni* or another species. Unraveling the natural history of *P. auduboni* from *P. marmoratus* from the extant literature will be difficult because locality data is often missing. Further collections with tissues for DNA work are necessary to fully document the diversity of these lizards.

Our examination of the morphology of three specimens from Pará, Brazil (MCZ 2889, 5549, 92644) agrees with the molecular results which suggest populations in Pará are neither *P. auduboni* nor *P. marmoratus*. These specimens have a higher vertebral count, more lamellae on the fourth finger and toe, and a loreal which contacts upper labials 3-4-5.

Additional taxa are likely present in Brazil's Atlantic Forest. *Polychrus virescens* Schniz (1822: 65) is likely a valid species. The type locality was not given by Schniz, but Wied-Neuwied (1825) considered the specimens to be *Polychrus marmoratus* when he published and reported the specimens as being from "Villa Viçosa am Flusse Peruhype." Vanzolini (1983: 119) noted this is now Nova Vicosá, Bahia, Brazil (-17.900872 -39.371644). Myers et al. (2011: 8) examined the history of this name and found Wagler (1828: pl 12) contained an illustration labeled *Polychrus virescens* and attributed the name to Wied-Neuwied in Schinz, but suggest it should be attributed to Schinz alone. They reason that Wied-Neuwied used *Polychrus virescens* as a manuscript name only and when he published referred the specimens to *Polychrus marmoratus*. We examined photographs of two specimens collected by Wied-Neuwied. An adult female has about 99 vertebrae which are keeled and some are multicarinate. Indicated femoral pores total 22, and the canthal contacts the loreal. The loreal is in three parts; it has one large scale between the supranasals (the largest scale on the snout); three scales separate the first canthals and four or five separate the second canthals; and the loreal contacts the supranasal. The semicircle scales are completely separated by a single row of scales.

Polychrus neovidanus Wagler (1833b: 897) is also a candidate for being a valid species. Vanzolini (1983) noted the description was based upon a figure in Seba (1734–1765, Volume 2, Plate 76, Figure 4) and on Spix's *P. marmoratus*. He states the name should be attached to Spix's specimen from Rio de Janeiro. We examined three specimens from Rio de Janeiro. They had 103–107 multicarinate paravertebrals and 70–88 scales around the mid-body. The canthal does not contact the loreal and the loreal contacts the supranasal. These specimens also differed from all other *Polychrus marmoratus* group members by having five scales between the first pair of canthals and seven scales between the second pair of canthals.

Hoogmoed (1973) Vanzolini (1983) and Avila-Pires (1995) suggest the *Polychrus marmoratus* group is disjunct with a population present in north and western South America, as well as with a second population in

the Atlantic Forest. However, Kawashita-Ribeiro and Ávila (2008) reported a specimen from Aripuanã in Mato Grosso which narrows the gap, suggesting the *P. marmoratus* group may be found throughout the area, and the disjunct distribution simply reflects a lack of collecting and knowledge.

Much remains to be learned about the *Polychrus marmoratus* group and how it reflects the historic landscapes of the South American continent. It seems likely that additional, unrecognized species remain to be discovered and we encourage further work on these remarkable and poorly studied lizards.

Acknowledgments.—Our sincerest thanks go to Harold K. Voris, Alan Resetar, and Kathleen Kelly at the Field Museum (FMNH); David Kizirian, and Lauren Vonnahme of the American Museum of Natural History (AMNH); Mike G. Rutherford at the University of the West Indies (UWIZM) and Greg Schneider at the University of Michigan, Museum of Zoology (UMMZ) for providing logistical support, access to the museum's collection, and data collection. For the loan of specimens and photography services we thank Ted Daeschler and Ned Gilmore at Academy of Natural Sciences (ANSP); Steve Rogers at Carnegie Museum (CM); Max Nickerson and Kenneth Krysko Florida Museum of Natural History (FLMNH); Jose Rosado at the Museum of Comparative Zoology; Smithsonian (USNM). We also thank Ana Prudente, Pedro Peloso, Gilson A. Rivas, Gabriel Ugueto, and Walter E. Schargel for photographs and discussions about *Polychrus*. Additionally appreciation goes to Alvin L. Braswell and Sara E. Murphy for comments on the manuscript.

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Appendix 1. Other specimens examined.

Skeletal and cleared and stained specimens examined: Ecuador: FMNH 42501; Peru: AMNH 71170 (Upper Ucayali), AMNH 71171 (Upper Ucayali); Suriname AMNH 141130, AMNH 148544 male AMNH 141084, UF 56618 (Guyana or Surinam), UF 60914 female, UF 68102. Trinidad: AMNH 148543 female, FMNH 49848, FMNH 49845, FMNH 49840, UF 18395, male, UF 18922. Venezuela FMNH 17791 (Sucre, Venezuela) male.

Polychrus liogaster ($n = 5$). Peru: FMNH 40586, 45499, 59184, 68599, 68600.

Polychrus neovidanus ($n = 3$). Brazil, Rio de Janeiro (-22.9 -43.23333) MCZ R-3390, R-170011, R-170012. *Polychrus virescens* Nova Vicosá, Bahai, Brazil (~17°54'15"S 39°22'W) AMNH R105, R1695. *Polychrus* sp. A ($n = 3$). Sucre Cocollar (10.147126 -63.808614) FMNH 17791–93.

Polychrus sp. B ($n = 3$). Brazil, Para (-1.45 -48.48333) MCZ R-2889, R-5549, R-92644.

Polychrus sp. C ($n = 4$) Bolivia: no specific locality BMNH 61.3.23.1; Buena Vista (-17.459161 -63.659221) FMNH 16163; 21510. Ecuador: FMNH 53890. *Polychrus* sp D. ($n = 1$) Venezuela, Bolivar (1.93965 -64.716248) UMMZ 85232.



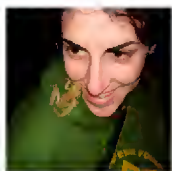
John C. Murphy resides in Sahuarita, Arizona and is a Research Associate at the Field Museum. His research interests focus on the herpetofauna of Trinidad and Tobago and aquatic snakes.



Richard M. Lehtinen is an Associate Professor, in the Department of Biology, The College of Wooster. His work on amphibians has taken him to Madagascar and Trinidad and Tobago.



Stevland P. Charles completed his Ph.D. at Howard University, in Washington D.C. in 2016. His research focused on the distribution, habitat and microhabitat use of the lizards in the genus *Gonatodes* native to Trinidad and Tobago, as well as the general effects of biogeography on the diversity of reptiles in Trinidad and Tobago. His current interests include the ecology, biogeography, behavior, systematics and conservation biology of Neotropical amphibians and reptiles.



Danielle Wasserman is a Ph.D. student at the City University of New York, studying trait evolution in avian and non-avian reptiles. She does collections based research and specializes in comparative morphology of vertebrates.



Tom Anton is president and CEO of the Ecological Consulting Group, LLC. A Chicago-based naturalist and historian he specializes in astacology (crayfishes), arachnology (specifically scorpions) herpetology and ichthyology. He is a Field Research Associate at the Field Museum, and an affiliate of the Illinois Natural History Survey.



Patrick Brennan graduated with a biology degree from The College of Wooster in 2013. He is currently working toward his Master's degree at the University of Toledo with a specialization in Bioinformatics.



Short Communication

New distribution records and conservation status of *Atelopus seminiferus* Cope, 1874: A Critically Endangered harlequin frog from northern Peru

¹Juan C. Cusi, ²Andy C. Barboza, ³Vance T. Vredenburg, and ⁴Rudolf von May

¹Departamento de Herpetología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Av. Arenales 1256, Jesús María, Lima, PERÚ ²División de Herpetología, CORBIDI (Centro de Ornitología y Biodiversidad), Santa Rita 117, Huertos de San Antonio, Surco, Lima, PERÚ ³Department of Biology, San Francisco State University, San Francisco, CA 94132-1722, USA ⁴Department of Ecology and Evolutionary Biology, University of Michigan, 1109 Geddes Ave, Ann Arbor, Michigan 48109–1079, USA

Abstract.—We provide information of the distribution, habitat, and conservation status of the harlequin frog *Atelopus seminiferus*, a poorly known species from northern Peru. Multiple individuals of *A. seminiferus* were detected inside the Alto Mayo Protected Forest, San Martín region, 87–98 km northwest from the type locality. Additionally, we used skin swab samples to test for the prevalence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), a pathogen that has been linked with population declines of harlequin frogs throughout tropical America. Our findings represent the first record of *A. seminiferus* inside a natural protected area, and we recommend an update of the IUCN Red List geographic range map of this species. Though we did not detect individuals infected by *Bd*, additional surveys are required to further assess the elevational distribution and potential for chytrid fungus infection of this Critically Endangered species.

Keywords. Bosque de Protección Alto Mayo, *Batrachochytrium dendrobatidis*, chytrid, UICN Red List, San Martín

Citation: Cusi JC, Barboza AC, Vredenburg VT, von May R. 2017. New distribution records and conservation status of *Atelopus seminiferus* Cope, 1874: A Critically Endangered harlequin frog from northern Peru. *Amphibian & Reptile Conservation* 11(1): 17–24 (e133).

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Received: 12 August 2016; **Accepted:** 22 November 2016; **Published:** 17 January 2017

Introduction

The Neotropical bufonid toad genus *Atelopus* contains 97 described species distributed across different habitats, from humid forest to paramo grassland in tropical America (Lötters 1996; Lötters et al. 2005). Of these, 69 species (71%) are categorized as Critically Endangered or Extinct under the IUCN Red List of Threatened Species (IUCN 2015). The conservation status of at least 30 species is uncertain because they remain undescribed (Coloma et al. 2010) or because a comprehensive systematic revision is required (La Marca et al. 2005; La Marca and Lötters. 2008; Lötters et al. 2011; Flechas et al. 2015). Peru contains 19 nominal species of *Atelopus* and three confirmed candidate new species from the Andes and Amazon regions (Frost 2016; Rueda-Almonacid et al. 2005). Of these, *A. loettersi*, *A. pulcher*, *A. spumarius*,

and *A. tricolor* are primarily distributed in the lowlands whereas the remaining species are restricted to elevations above 1,000 m. Montane areas along the eastern slopes of the Andes are particularly important habitat because they harbor many species of *Atelopus*. Although several species have not been seen in decades, recent field surveys have uncovered rare species such as *A. epikeisthos* (R. Santa-Cruz et al., In press). Because of this, it is essential to continue surveying these montane areas to assess if amphibian species, some of which have not been seen in many decades (e.g., Lehr and von May 2004), still exist, and to evaluate their current conservation status.

Atelopus seminiferus was described by Cope in 1874 based on a single specimen (ANSP 11383) collected by Prof. Orton from between Balsa Puerto and Moyobamba, San Martín department, northern Peru (Malnate 1971). Subsequently, this species was recorded at the Quebra-

Correspondence. Emails: jcarloscusim@gmail.com; rvmay@gmail.com

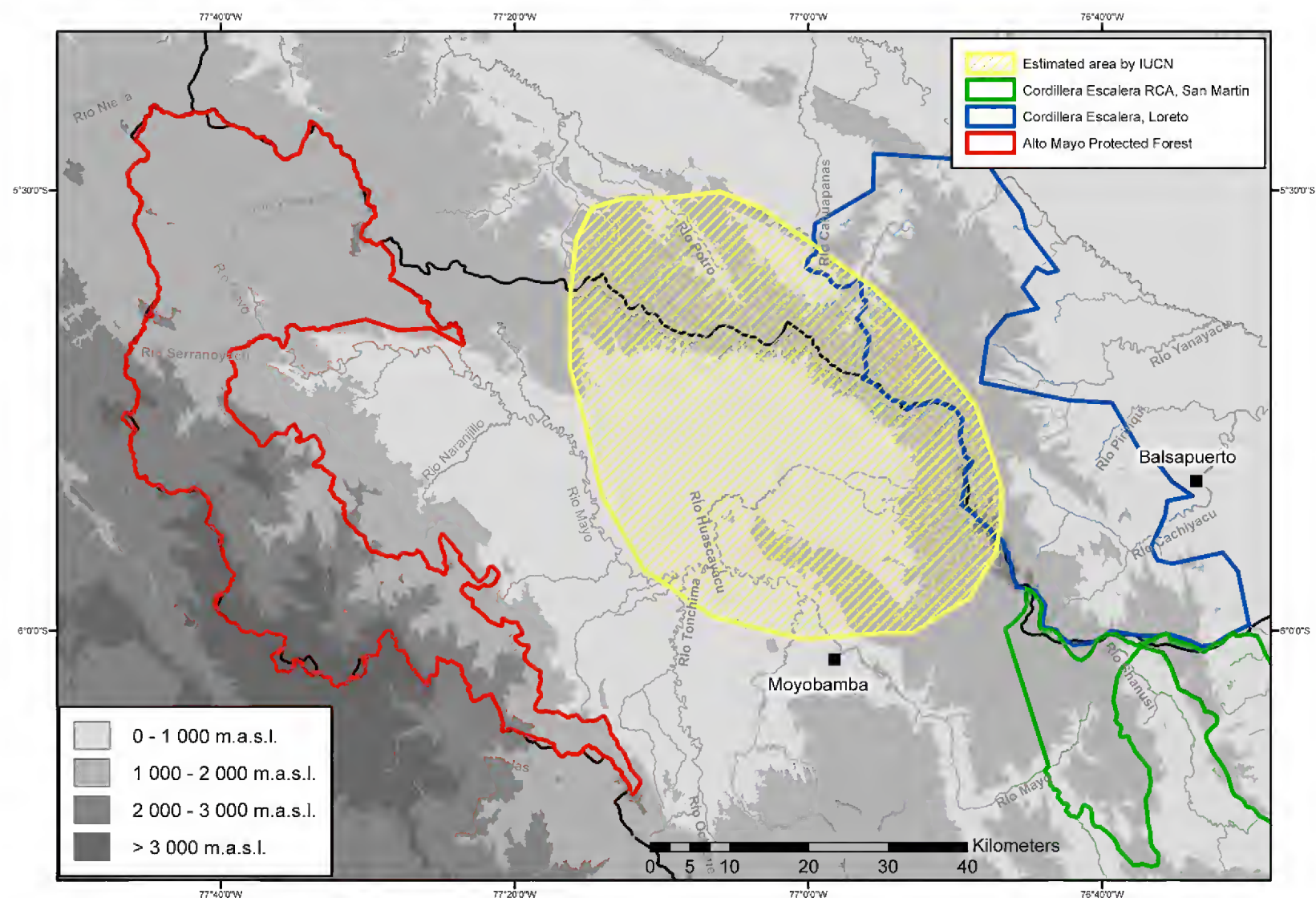


Fig. 1. Distribution of *Atelopus seminiferus* in the Mayo River basin, San Martín, Peru. Yellow polygon corresponds to geographic range estimated by IUCN. Compare with Fig. 3, which shows proposed new polygon based on results from this study. Map by Juan C. Cusi.

da Kevlada, close to an Awajun native village in Rioja Province, San Martín (Schulte et al. 2004; R. Schulte, pers. comm.). This second record was near an urban area known as Naranjos, located along the Fernando Belaunde road (05°44'34.05"S, 77°30'20.87"W, 959 m) and ca. 7.5 km E from the boundary of Alto Mayo Protected Forest (AMPF). Subsequently, AMPF park rangers reported this species in 2007 from a site between Nuevo Eden-El Carmen villages, Rioja, San Martín (although no geographic coordinates available). In 2008, biologist Jorge Carrillo conducted field research focused on harlequin frogs (*Atelopus* spp.) at Sector Serranoyacu inside the AMPF, but no specimens were recorded in this area (J. Carrillo, pers. comm.). Recent herpetological surveys at the AMPF indicate that this reserve has at least 35 species of amphibians and 10 species of reptiles (J. Cusi et al., unpubl. data). Prior to this study, which we present here, no records of *A. seminiferus* were available from the AMPF. Thus, presenting new data on *A. seminiferus* is relevant given that recent studies of threatened amphibians from Peru did not include this Critically Endangered species (e.g., von May et al. 2008; Jarvis et al. 2015).

In this report, we provide new distributional data for *A. seminiferus* and recommend an update to the map of its known geographic distribution (Fig. 1). We also tested for the prevalence of the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), a pathogen that has been linked with population declines of harlequin frogs throughout tropical America (Lampo et al. 2006; Venegas et al.

2008; Flechas et al. 2015). Additionally, given that other factors such as habitat loss may have caused population declines in many other amphibian species (Catenazzi and von May 2014; Tarvin et al. 2014), we noted the type of habitat used by *A. seminiferus* in the region. Although conducting a thorough assessment of habitat change and disturbance was not a goal of the study, we provide preliminary information about habitat change and disturbance observed at some localities.

Methods and Materials

We conducted fieldwork at the Alto Mayo Protected Forest (AMPF) and Moyobamba, San Martín region, between March and December 2014 (Fig. 1). The AMPF is located along the Cordillera Oriental and is part of the upper basin of the Mayo River, northern Peru. Additionally, we surveyed around the city of Moyobamba because it is one of the type localities of *A. seminiferus*. A team of 2–3 people carried out Visual Encounter Surveys (Angulo et al. 2006; Crump and Scott 1994) during both diurnal (10:00–14:00) and nocturnal periods (18:00–00:00). Total survey effort at AMPF was 240.5 person-hours, and 16.2% of it (39 person-hours) was invested at El Carmen village, within the Venceremos sector. We collected life history data including sex, snout-vent length, and weight, as well as the type of substrate used by each individual. Additionally, we collected skin swab samples to test for prevalence of the chytrid fungus *Batrachochytrium*

Table 1. Known localities for *Atelopus seminiferus* in the basin river Mayo, San Martin, Peru. Total survey effort around El Carmen village (all localities combined) was 39 person-hours.

Locality	Map locality number	Latitude	Longitude	Elevation	Date	No. Ind.	Sex	Reference
Kevlada creek, km 393.7, near the Naranjos village	1	—	—	1100	2004	2	—	Rainer Schulte (Second record)
Trail between El Carmen and La Esperanza villages	2	5°38'48.5" S	77°41'26.0" W	1641	18/01/14	2	♂, ♀	Fredi Sangama and Florencio León*
El Arenal forest, near El Carmen village	3	5°36'26.6" S	77°41'33.1" W	1279	23/08/14	1	♀	Authors
Nueva Jordania village	4	5°34'51.2" S	77°40'50.7" W	1127	Nov. 2011	1	—	Mathieu Chouteau
El Carmen village: coffee plantations	5	5°34'57.7" S	77°41'38.2" W	1134	30/06/14	1	♀	Authors
El Carmen village	6	5°35'30.4" S	77°42'05.5" W	1243	6/10/13	1	—	Florencio León*
El Carmen village	6	5°35'30.3" S	77°42'02.1" W	1224	26/08/14	1	—	Jhonny Ramos and Elan Cachique*
El Carmen village	6	5°35'30.4" S	77°42'03.3" W	1222	30/06/14	1	♂	Authors
El Carmen Creek	6	5°35'36.2" S	77°42'06.7" W	1277	22/08/14	1	♀ gravid	Authors
El Carmen Creek	6	5°35'36.7" S	77°42'07.2" W	1267	22/08/14	1	♂	Authors
El Carmen village	6	5°35'29.1" S	77°42'03.3" W	1229	1/07/14	1	♀	Authors
Las Palmas village	7	5°37'43.6" S	77°43'57.0" W	1902	16/10/13	2	—	Florencio León*
Villa Hermosa village (Boundary Amazonas-San Martín)	8	5°32'32.4" S	77°45'49.9" W	1756	17/03/14	1	—	Marco Ramirez*

* Park rangers' names (Alto Mayo Protected Forest)

dendrobatidis (Bd). We took skin tissue samples using MW113-Advantage Bundling sterile cotton swabs over the abdomen, thighs, and hind limbs of each animal for a total of 30 strokes (Catenazzi et al. 2013). Skin swab samples were stored in 1.5 ml tubes. DNA was extracted from each swab and analyzed following standard protocols (Boyle et al. 2004; Hyatt et al. 2007). Additionally, we used a handheld infrared thermometer (RayTek MiniTemp MT6) to record the body temperature in vivo and the temperature of the substrate used by individuals of *A. seminiferus*. Air temperature and relative humidity were measured every 15 minutes during 24 hours with one HOBO U23 Pro v2 data logger (Onset) at one of the survey sites. Given that, at the time of the study, only the holotype was available in a museum collection (Academy of Natural Sciences, Philadelphia [ANSP] in North America), four specimens were collected (MUSM 33328, 33327, 33662, JCM H-24) and deposited as reference material at the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM) in Lima, Peru. For this purpose, a research and collecting permit (RJ N° 001-2014-SERNANP-BPAM-JEF) was obtained from Peru's Ministerio del Ambiente.

Results

We found individuals of *A. seminiferus* at six localities within of the Alto Mayo Protected Forest: 1) trail between El Carmen and La Esperanza villages, 2) El Arenal forest near El Carmen village, 3) Nueva Jordania village, 4) El Carmen village, including the actual village, a creek, and coffee plantations, 5) Las Palmas village, and 6) Villa Hermosa village (Table 1). All of these localities fall outside the IUCN range map polygon (<http://>

maps.iucnredlist.org/map.html?id=54548, accessed on 8 August 2016; Fig. 1). Most individuals observed in the field were detected around El Carmen, a small village inhabited primarily by coffee farmers and surrounded by coffee plantations. We also obtained photographic records from Las Palmas and Villa Hermosa, provided by park rangers who found *A. seminiferus* during patrols and surveillance against illegal logging of the forests. Even though we did not visit Nueva Jordania, Mathieu Chouteau (pers. comm.) informed us about the record of one specimen in this locality (Table 1). The complete set of these new localities was used to calculate the extent of occurrence. Using ArcGIS, we estimated that the Extent of Occurrence of *A. seminiferus* is ca. 2,520 km².

We recorded 14 adult individuals at the AMPF between March and December on 2014. Most adult individuals were found on leaf litter between 10:00 h to 14:00 h (MUSM 33328, 33327, 33662), and some individuals were found at night along the margins of a creek; these individuals were sitting on top of fern leaves near the ground. Although we did not hear vocalizations of *A. seminiferus*, one mating pair was photographed on 18 January 2014 (Fig. 2A) and one gravid female (field number JCM H-24) was found on 22 August 2014. Advertisement calls and tadpoles of this species remain unknown. Coloration pattern coincides with the description of Lötters and Schulte (2005): dorsal surface uniformly velvety black with minute yellow or pinkish cream dots scattered throughout the dorsum, forelimbs and hindlimbs; some individuals possess pinkish dots on lower jaw; palmar and plantar surfaces dark red; belly pink in males and dark red in females, over a black background in both sexes; throat with pink blotches over a black background; iris black with yellow ring around pupil (Fig. 2 B–D).

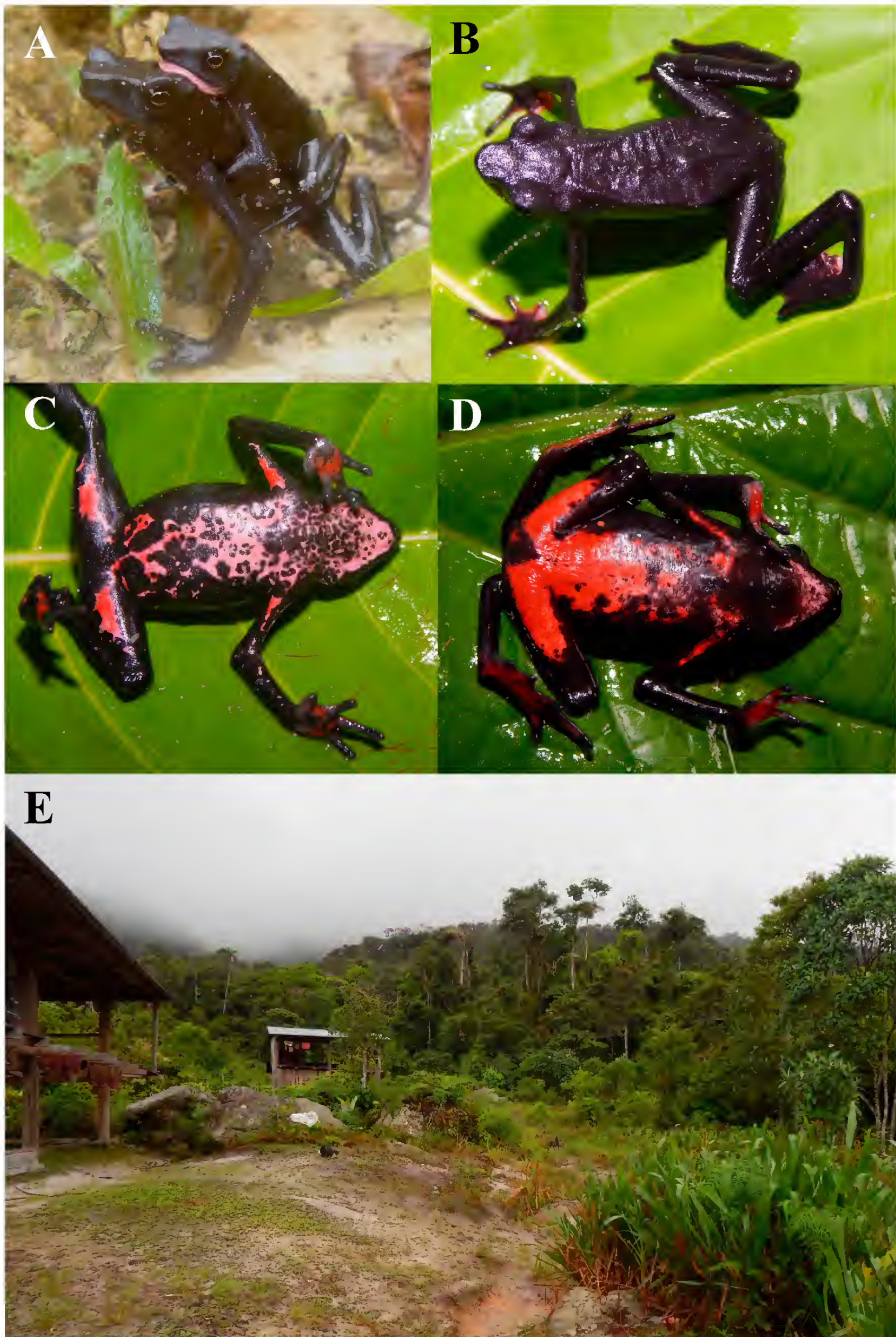


Fig. 2. (A) A pair of *Atelopus seminiferus* in amplexus, found between El Carmen and La Esperanza [not collected]. Photo by Fredi Sangama and Florencio León. (B) Dorsal coloration pattern of a female MUSM 33328. (C) Ventral coloration pattern in a male MUSM 33327. (D) Ventral coloration pattern in a female MUSM JCM H-24. (E) El Carmen village in Alto Mayo Protected Forest, Rioja province, San Martín (E). Photos B–E by Juan C. Cusi.

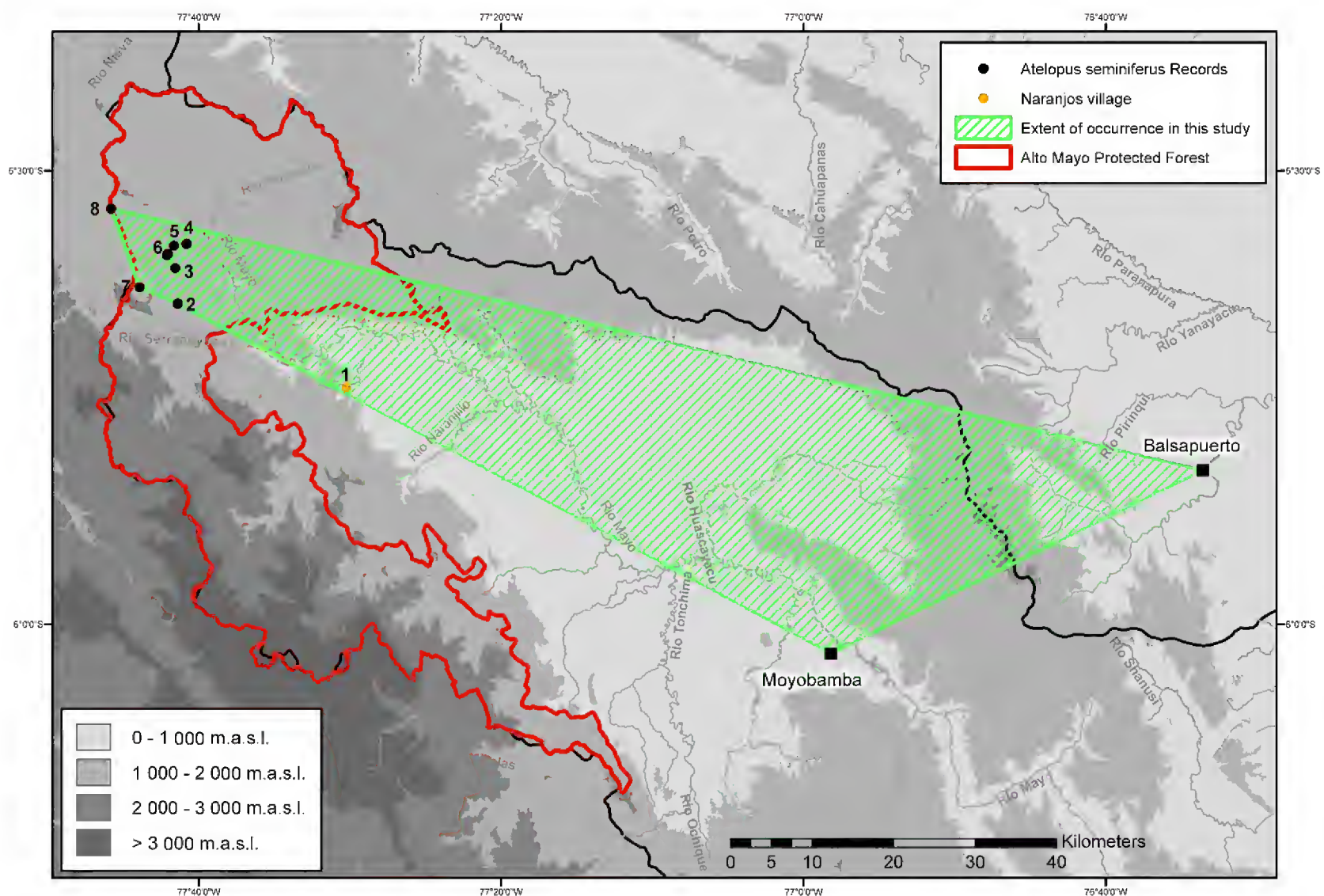


Fig. 3. Updated distribution map of *Atelopus seminiferus*. Black dots indicate new localities reported in this study. Light green area corresponds the estimated Extent of Occurrence (ca. 2,520 km²) based on the new records presented here and the previously known localities. Numbers correspond to labels in Table 1. Map by Juan C. Cusi.

Our *Bd* prevalence assays were negative for the presence of the chytrid fungus (*Bd*) in samples from El Carmen (*Bd* negative, $n = 5$). Mean body temperature was 19.9 °C, and temperatures of air and substrate were 21.2 °C and 21.3 °C ($n = 4$), respectively. We recorded climatic parameters in one primary forest near El Carmen (locality number 6 in Fig. 1 and Table 1) on 27–28 June 2014. Mean air temperature during the day was 18.48 ± 2.14 °C and relative humidity was $96.89 \pm 2.67\%$. Mean air temperature at night was 15.90 ± 1.19 °C and relative humidity was $98.16 \pm 2.41\%$. Using the new records and the previously known localities, we created a polygon to update the geographic distribution map of *A. seminiferus* (Fig. 3).

Discussion

Our study documents the existence of populations of *A. seminiferus* inside the Alto Mayo Protected Forest, and it represents the first record of this Critically Endangered species inside a natural protected area. The new localities reported here represent an extension of the geographic range of *A. seminiferus* by ca. 45 km west from the western boundary of the geographic range recognized by IUCN (Fig. 1). Specifically, the new localities are ca. 23.1 km northwest from Naranjos and 86.7 km northwest from Moyobamba (type locality). The record from Villa Hermosa (Table 1) represents the northernmost locality known to date for this species. Therefore, we recommend

an update of the IUCN Red List geographic range map of this species. Concretely, we recommend that the new polygon generated here (Fig. 3) should be considered in the next IUCN Red List assessment and replace the currently available polygon. As with most harlequin frogs, *A. seminiferus* is considered a rare species given that very few specimens have been observed and collected in the wild. The IUCN Red List assessment (Schulte et al. 2004) states that data on population status or abundance were not available and emphasized that additional field surveys were needed in the region. In addition to detecting *A. seminiferus* at six new localities (i.e., sites located >1 km apart from each other), our findings suggest that this species has a fragmented distribution. Using the IUCN Red List criteria (IUCN 2016), which indicates that if a species is known from fewer than ten threat-defined locations and the extent of occurrence is smaller than 20,000 km², it should be classified as Vulnerable or Endangered. *Atelopus seminiferus* is known from eight localities (Table 1), has an estimated Extent of Occurrence (EOO) of 2,520 km²; additionally, the estimated EOO and the number of known subpopulations or locations has varied over time (with a total of 16 individuals detected in 10 years). Therefore, we suggest that *A. seminiferus* might be classified as Vulnerable B1ac(i,iii).

Our field surveys indicate that *A. seminiferus* inhabits primary montane forests and might tolerate some level of disturbance given that some individuals were found in modified forested habitats. In particular, *A. seminiferus*

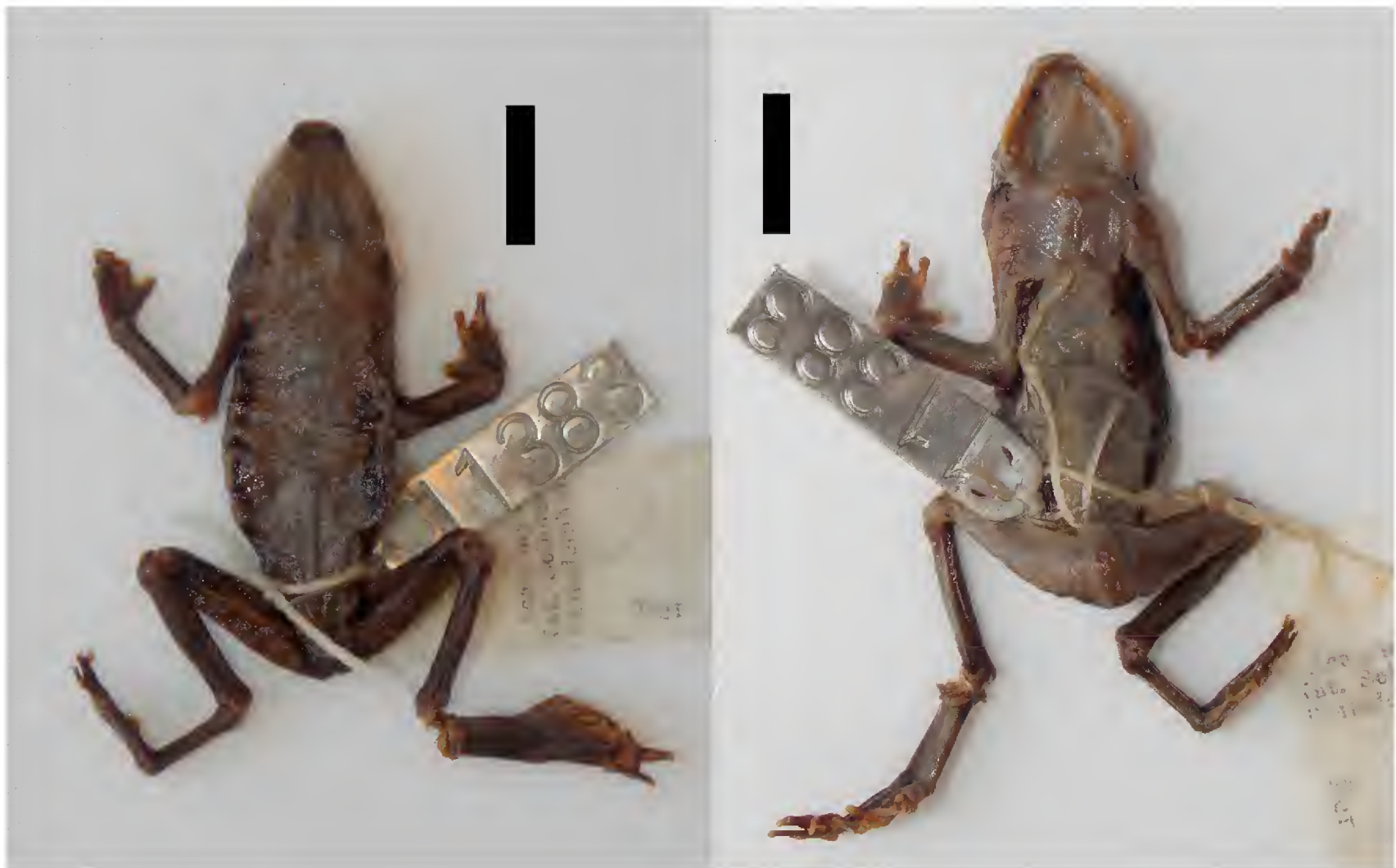


Fig. 3. Dorsal and ventral views of the holotype of *Atelopus seminiferus* (ANSP 11383), deposited in the herpetological collection at the Academy of Natural Sciences of Drexel University, Philadelphia. Photos courtesy of Ned Gilmore.

occurs in areas surrounding El Carmen (Fig. 2E), where native montane forests have been cleared and replaced by subsistence agricultural plantations (coffee, pineapple, and banana) and areas used by livestock (cattle, horses, and mules). However, it would be premature to assume that populations of *A. seminiferus* in disturbed areas will persist on the long term, given that pesticides and fertilizers used in agricultural plantations may have negative effects on amphibians (Hayes et al. 2002). Furthermore, habitats used by *A. seminiferus* appear to be impacted by the expansion of human settlements associated with urban development in Moyobamba and Balsapuerto in recent years. Given the geographic proximity to Moyobamba-Balsapuerto, it is possible that *A. seminiferus* also occurs at Cordillera La Escalera Regional Conservation Area (RCA), in San Martín region, and Cordillera Escalera, in Loreto region; Fig. 1. However, the species has not been detected in this protected area (Pitman et al. 2014). The chytrid fungus (*Bd*), a pathogen associated with massive declines of amphibians around the world (Catenazzi et al. 2011; Lips et al. 2008; Vredenburg et al. 2010), has been assumed to be a possible threat for *A. seminiferus*. Although we did not detect *Bd*-infected individuals, further monitoring of populations of *A. seminiferus* and larger skin swab sample sizes are needed to test if the pathogen is affecting any of these populations more widely. Continuous assessment of *Bd* prevalence is essential given that chytridiomycosis has likely affected many species of *Atelopus* (Bonaccorso et al. 2003; La Marca et al. 2005; Lampo et al. 2006; Lips et al. 2008). The new voucher specimens collected during this study will be useful for future morphological studies, especial-

ly because the only available type material (the holotype, ANSP 11383) has deteriorated and has broken phalanges on left hand and right foot (Fig. 4). In summary, our findings provide valuable insights on the conservation status of *A. seminiferus* and an updated map of the known geographic range of this species.

Acknowledgments.—We thank the Ministry for Foreign Affairs of Finland and Conservation International Foundation (BioCuenas project) for financing our research. We thank Dr. Ulla Helimo for her encouragement and valuable suggestions on our research plan; Jesús Córdova and Betty Millán for providing access to Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM), Peru. We also thank Rainer Schulte and Stefan Lötters for providing information and suggestions for the manuscript. Gustavo Montoya and Ivonne Paico of the AMPF office kindly helped with collecting permits (RJ N°001-2014-SERNANP-BPAM-JEF) and park rangers (Jhonny Ramos, Florencio León, Fredi Sangama, Marco Ramírez, Elan Cachique) that gently provided records of this species of *Atelopus*, and we recognize their valuable efforts for the conservation of the forests in Alto Mayo region. We also thank Rainer Schulte and Mathieu Chouteau for kindly providing locality information on *A. seminiferus*, and Ned Gilmore (Academy of Natural Sciences of Drexel University, Philadelphia) for kindly providing photos of the holotype of *A. seminiferus*. Thanks to Mr. Bartolomé (local guide) at El Carmen for its assistance in field and hospitality in his property. We thank two anonymous reviewers for providing helpful comments on the manuscript.

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Juan Carlos Cusi is an associate researcher at the Herpetology Department at the Museum of Natural History, Universidad Nacional Mayor de San Marcos, Perú (MUSM). His research interests include the taxonomy and ecology of amphibian and reptiles, and he is currently completing a Master's program in zoology at the Universidad Nacional Mayor de San Marcos. His thesis focuses on molecular phylogenetics and morphology of Neotropical salamanders in the genus *Bolitoglossa*.



Andy C. Barboza is a Peruvian biologist and scientist associated with the Herpetological Collection of Centro de Ornitología y Biodiversidad (CORBIDI), Peru. Her research interests focus on systematic and evolutionary history of amphibians and reptiles of the Neotropical region.



Vance T. Vredenburg is an Associate Professor in the Department of Biology at San Francisco State University, research associate and fellow of the California Academy of Sciences, and research associate at the Museum of Vertebrate Zoology at UC Berkeley. His current research focuses on the impacts of emerging infectious diseases on amphibians (e.g., chytridiomycosis) and the role of the amphibian skin microbiome in health and disease. He is also co-founder of AmphibiaWeb (www.AmphibiaWeb.org), an online conservation resource for amphibians.



Rudolf von May is a postdoctoral research fellow at the Department of Ecology and Evolutionary Biology at the University of Michigan. His current research seeks to understand how amphibian and reptile communities are structured across habitats and elevations, taking into account the phylogenetic relatedness among species present in those communities.



Restricted diet in a vulnerable native turtle, *Malaclemys terrapin* (Schoepff), on the oceanic islands of Bermuda

^{1,3,4}Mark E. Outerbridge, ²Ruth O’Riordan, ²Thomas Quirke and ²John Davenport

¹Department of Environment and Natural Resources, 17 North Shore Road, Hamilton Parish, FL04, BERMUDA ²School of Biological, Environmental and Earth Sciences, University College Cork, Distillery Fields, Cork, IRELAND

Abstract.—Diamondback Terrapins (*Malaclemys terrapin*) are native to Bermuda, presently inhabiting only four small brackish-water ponds. Their foraging ecology was investigated using direct observation, fecal analysis, and necropsy. They do not have as varied a diet as reported from their North American range. Small gastropods (<3 mm shell height) were found in 66.7% of fecal samples and made up 97.3% of animal material dry mass, thus dominating their diet. Scavenged fish and other vertebrates (19% of samples overall), plus terrestrial arthropods (14.3% of samples) were other common items. Polychaete worms and bivalves each occurred in less than 3% of fecal samples. Pond sediment was found in 74% of the samples, probably incidentally ingested while foraging (by oral dredging) for the gastropods. The distribution and abundance of arthropods and molluscs within the terrapins’ brackish-water environment were assessed in three different habitats; pond benthos, mangrove swamp, and grass-dominated marsh. These indicated that Bermuda’s terrapins do not fully exploit the food resources present. On Bermuda *M. terrapin* is basically a specialist microphagous molluscivore and mainly forages by deposit-feeding on gastropods living in soft sediments. This dietary restriction has made them particularly vulnerable to environmental contamination.

Keywords. Anchialine pond, Diamondback Terrapin, fecal analysis, feeding ecology, aquatic gastropod

Citation: Outerbridge ME, O’Riordan R, Quirke T, Davenport J. 2017. Restricted diet in a vulnerable native turtle, *Malaclemys terrapin* (Schoepff), on the oceanic islands of Bermuda. *Amphibian & Reptile Conservation* 11(1): 25–35 (e134).

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Received: 29 March 2016; **Accepted:** 08 September 2016; **Published:** 26 January 2017

The Diamondback Terrapin *Malaclemys terrapin* is one of two emydid turtle species living in the inland pond environments of the oceanic islands of Bermuda. The other, *Trachemys scripta elegans*, is a widely-distributed introduced freshwater pest (Outerbridge 2008). Diamondback Terrapins are less abundant than the sliders and have a greatly restricted local brackish distribution (Davenport et al. 2005). Native to Bermuda (Davenport et al. 2005; Parham et al. 2008) they form the only known population outside of the USA.

Diamondback Terrapins have been identified as an important component of the trophic dynamics of the east coast USA salt marsh ecosystem (Silliman and Bertness 2002; Davenport 2011) and are carnivorous, feeding mostly upon a variety of marine molluscs and crustaceans throughout the North American range (Butler et al. 2006; Ernst and Lovich 2009). There is, however, a growing body of evidence to support the hypothesis that this

terrapin species may be a dietary generalist that is opportunistic in its foraging habits (Spivey 1998; Petrochic 2009; Butler et al. 2012; Erasmus 2012). Diamondback Terrapins show resource partitioning, whereby individuals with wider heads (the largest females) consume larger snails and crabs than terrapins possessing smaller heads (Tucker et al. 1995). Diamondbacks appear to be predators that use visual cues while foraging, showing selectivity in the prey that they eat (Davenport et al. 1992; Tucker et al. 1995, 1997; Butler et al. 2012).

Though the diet of Diamondback Terrapins has been studied in various regions throughout their North American range, no studies have been conducted on Bermuda. Analysis of fecal material is a non-destructive and non-invasive way of examining dietary preference and has been used on several species of small turtles previously (Demuth and Buhlmann 1997; Lima et al. 1997), including Diamondback Terrapins (Tucker

Correspondence. ³mouterbridge@gov.bm. Present address: ⁴P.O. Box FL 145, Flatt’s Village, FL BX, BERMUDA

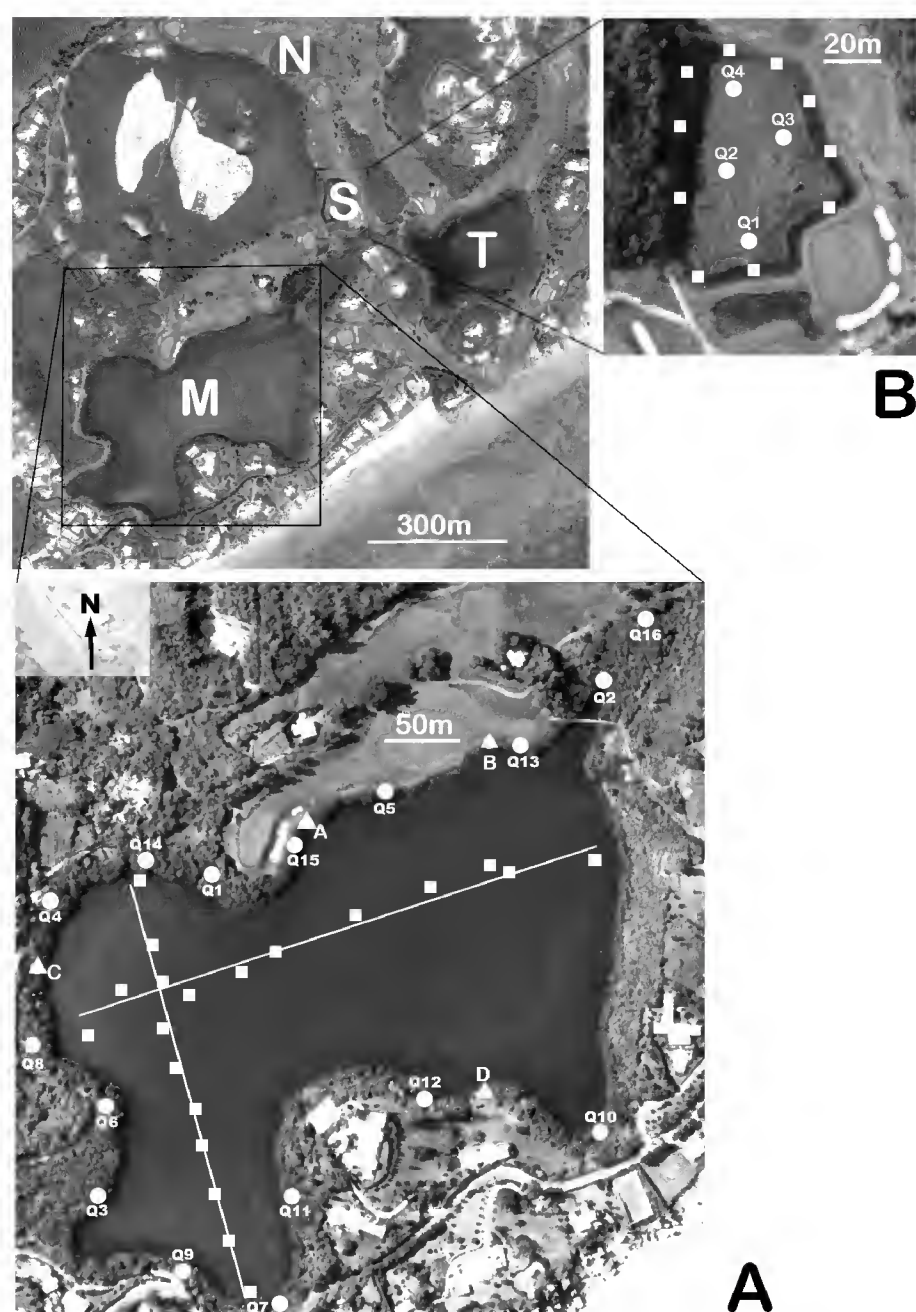


Fig. 1. Benthic survey locations in Mangrove Lake (A) and South Pond (B). Squares represent detritus sample locations along the belt transects; triangles represent the pond quadrat sample locations; circles represent the quadrat sample locations in the adjacent wetland communities. M = Mangrove Lake, T = Trott's Pond, S = South Pond, N = North Pond.

et al. 1995; Spivey 1998; Roosenburg et al. 1999; King 2007; Petrochic 2009; Butler et al. 2012; Erasmus 2012; Tulipani 2013; Tulipani and Lipcius 2014). This method of dietary determination has the added benefit of allowing multiple samples to be taken from a single individual over time. However, it is limited by the differential digestibility of the various hard and soft-bodied dietary components which in turn affects their representation within the feces.

The primary objective of the current investigation was to examine the diet and foraging ecology of Bermuda's terrapin population, with specific aims to assess food preferences within the land-locked, brackish-water pond environment, as well as assess the abundance and distribution of potential food items within the ponds and adjacent wetland communities. It was envisaged that detailed knowledge of terrapin diet in Bermuda would help appropriate conservation and management efforts to be directed towards protecting the areas in which they forage.

Materials and Methods

Study Site

Bermudian Diamondback Terrapins occur in four neighbouring brackish-water ponds: Mangrove Lake, South Pond, North Pond, and Trott's Pond (Fig. 1) situated on a private golf course located at the eastern end of the islands (32.32858°N, 64.70547°W; WGS 84). They move between these ponds (Outerbridge 2014). Mangrove Lake (10 ha area) and Trott's Pond (3 ha) are the largest of these and both are simple, shallow, anchialine basins fringed by Red Mangrove Trees (*Rhizophora mangle*) with deep benthic deposits of highly organic sediment (Thomas et al. 1992). Anchialine ponds are relatively small land-locked brackish bodies of water with subterranean connections to the sea (Holthuis 1973) and they show limited tidal influence. North Pond (0.4 ha) and South Pond (0.5 ha) are considerably smaller in area, shallower in depth, and lack mangrove vegetation. However, both have small central marshes dominated by grasses (*Cladium jamaicense* and *Paspalum vaginatum*). All ponds were incorporated into the golf course as water hazards during the 1920s, are situated upon a single square kilometer of land, and are only separated from each other by, at most, 380 m of land (straight-line distance between North Pond and Trott's Pond).

Fecal Analyses

Juvenile, immature, and adult Diamondback Terrapins were opportunistically captured using a long-handled dip net from Mangrove Lake, South Pond, North Pond, and Trott's Pond from March–September 2010 and January–October 2011. Maturity status was determined following Lovich and Gibbons (1990); individuals <91 mm straight plastron length (SPL) were classified as juveniles, males as sexually mature if $SPL = 91–137$ mm, females as sexually mature if $SPL \geq 138$ mm. Females of SPL 91–137 mm were regarded as immature. After capture, each individual was kept outside in the shade for 48 h in covered, plastic storage bins (55 cm long \times 45 cm wide \times 30 cm deep). All fecal material collected in the 48 h period was strained through a one mm mesh-sized sieve, oven dried at 80 °C for 48 hours, and stored in a sealed glass vial for subsequent identification. Fecal samples were also collected from neonate terrapins (i.e., individuals that were less than one year old) that were followed as part of a radio-telemetry study (Outerbridge 2014). At the end of the tracking period, each individual was placed in a 500 ml plastic bowl containing enough freshwater to cover the carapace and held in a room with an ambient temperature of 30 °C for 48 hours. All fecal material collected in this period was strained through 47 mm filter paper to retain finer particles from smaller prey items consumed, allowed to air dry for 48 hours,

and stored in a sealed glass vial. All terrapins captured during the fecal analysis investigation were released at their original capture location.

Each fecal sample was examined at magnifications between 10× and 25× using a stereoscopic microscope with an ocular scale. Food items were identified to the lowest possible taxonomic level, and weighed to the nearest 0.0001g. The shells of gastropods, when encountered whole, were counted and shell height (SH; maximum measurement along the central axis) was measured to the nearest 1.0 mm (note that some fecal samples only contained broken shells, the size of which could not be estimated). Quantification of dietary items was accomplished by determining the percentage dry mass of each item relative to the total dry mass of each sample. The relative frequency of occurrence of each dietary item was determined by calculating the percentage of turtles containing a given food type in relation to the total number of turtles examined.

Benthic Biotic Surveys within the Terrapins' Wetland Environment

Assessments of mollusc and crustacean abundance and distribution within the ponds and adjacent wetland environments were conducted to determine prey availability for Bermuda's Diamondback Terrapins. These assessments were accomplished by performing a series of benthic transects within three different habitats utilized by all size and age classes of Bermuda's Diamondback Terrapins; the sediment at the bottom of Mangrove Lake and South Pond, the Red Mangrove swamp community that surrounds Mangrove Lake, and the Saw-grass (*Cladium jamaicense*) marsh in the center of South Pond.

Pond Benthic Surveys

Two belt transect surveys of benthic biota were performed in Mangrove Lake and one belt transect survey was carried out in South Pond in July 2011. The Mangrove Lake transects were straight-line and followed an east-west direction (Transect 1) and a south-north direction (Transect 2), whereas the survey in South Pond was circular (Transect 3). Ten locations were haphazardly sampled along the path of each transect (Figs. 1A, 1B). The GPS coordinates were recorded at each location together with a brief description of the benthic characteristics. Collection consisted of sweeping a dip net with one mm mesh and a square opening of 25 x 25 cm for a distance of one m and a depth of approximately 2.5 cm at the surface of the sediment (thereby sampling a linear area of 0.25 m² at each location). The collected sediment was passed through a one mm mesh sieve at the surface of the pond and the material that remained was transferred into a one litre container. In addition to the belt transects, four replicate 25 x 25 cm quadrat surveys (A–D, Fig. 1A) were performed at random in sand, rock, and gravel areas of the margins of Mangrove Lake. The

area defined by each quadrat was dredged to a depth of 2.5 cm and the contents transferred into a bucket and sorted by hand.

Mangrove Swamp Surveys

Sixteen replicate quadrat surveys were performed within the mangrove swamp that borders Mangrove Lake (Q1–Q16, Fig. 1A). The sites were haphazardly chosen, using an aerial map, at various locations around the periphery of the pond. Upon arrival in the field, a 25 × 25 cm quadrat was randomly placed upon the leaf litter immediately land-ward of the water-line. The area defined by each quadrat was dug to a depth of 2.5 cm and the contents transferred to a 3.8 liter sealable plastic bag. The contents of each bag were gently sifted in the laboratory using running water and a sieve with five mm mesh stacked on top of a one mm mesh-sized sieve.

Saw-grass Marsh Surveys

Four replicate quadrat surveys were performed within the saw-grass marsh at the center of South Pond (Q1–Q4, Fig. 1B). These sites were also haphazardly chosen using an aerial map. Upon arrival in the field, a 25 × 25 cm sample of saw-grass and turf was cut, to a depth of 2.5 cm, from the marsh at each of the four sites. The saw-grass blocks were transferred to separate 19 L buckets and taken to the laboratory for examination. Each sample was placed in a plastic bin (60 cm long × 40 cm wide × 14 cm deep), carefully broken apart and gently sifted in the laboratory using running water and a five mm sieve stacked on top of a one mm sieve. Shoot bundles were counted to determine saw-grass density.

All biological specimens from the belt transect and quadrat surveys were kept for subsequent identification in the laboratory, but only living specimens were counted and measured (i.e., empty gastropod shells were discarded). Live gastropods were counted, measured (total shell height mm), and frozen for eco-toxicological analyses (Outerbridge et al. 2016). All other living biological specimens were returned to their original locations and released after identification. All transect and quadrat survey results were standardized as values m⁻² as depth was constant throughout.

Results

Fecal Analyses

A total of 54 Diamondback Terrapins were netted between March and September 2010 ($n = 21$) and January and October 2011 ($n = 33$), of which 42 (77.8%) produced fecal samples during the 48-hour confinement period (30 adults, four immature females, three juveniles of undetermined gender, and five neonates). Of the 54 terrapins, 30 were captured from South Pond (of which 23 (76.7%) produced fecal samples), 20 from Mangrove Lake (of

Table 1. *Malaclemys terrapin* dietary items obtained from 42 fecal samples (from females, males, juveniles and neonates combined) collected from inhabitants of four brackish ponds in Bermuda. Symbols: n = number of samples containing a given food type; % = percentage of samples containing a given food type in relation to the total number of samples. Presence (+) and absence (-) of dietary items' data for the various gender/age categories are given separately.

Dietary Item	n (%)	Adult females	Adult males	Juveniles	Neonates
Plants (grass, seeds, algae)	14 (33.3%)	+	+	+	-
Gastropoda	28 (66.7%)	+	+	+	+
<i>Heleobops bermudensis</i>	24 (57.1%)	+	+	+	+
<i>Melanoides tuberculata</i>	15 (35.7%)	+	+	+	-
<i>Melampus coffeus</i>	2 (4.8%)	+	-	-	-
Insecta	6 (14.3%)	+	-	+	+
Polychaeta					
<i>Arenicola cristata</i>	1 (2.4%)	-	+	-	-
Bivalvia					
<i>Isognomon alatus</i>	1 (2.4%)	+	-	-	-
Crustacea					
<i>Armadillidium vulgare</i>	1 (2.4%)	+	-	-	-
Osteichthyes					
<i>Fundulus bermudae</i>	5 (11.9%)	+	+	-	-
Amphibia/Reptilia					
<i>Rhinella</i> (syn <i>Bufo</i>) <i>marinus</i>	2 (4.8%)	+	+	-	-
<i>Malaclemys terrapin</i>	1 (2.4%)	+	+	-	-
Sediment	31 (73.8%)	+	+	+	-
Trash (cigarette filter)	1 (2.4%)	+	-	-	-

which 15 [75.0%] produced fecal samples), three from North Pond (all of which produced fecal samples), and one was captured from Trott's Pond (which also produced a fecal sample). Note that the small Bermudian terrapin population meant that some terrapins were netted more than once in this exercise; three females, one male, and one neonate were captured twice. One of the females was captured three times.

Of the 42 terrapins that produced fecal matter, 28 (66.7%) were classified as female (24 mature, four immature) ranging from 126–196 mm straight carapace length (SCL) (mean 172, SD 17.9) and six (14.3%) were classified as male (all mature) ranging from 114–134 mm SCL (mean 122, SD 8). Three (7.1%) were classified as juveniles (97–107 mm SCL, mean 102, SD 5), and five (11.9%) were classified as neonates (31–35 mm SCL, mean 33.7, SD 1.6).

Sediment occurred in 73.8% of the fecal samples, gastropods in 66.7%, plant material in 33.3%, fish and other vertebrate bones in 19%, terrestrial arthropods in 14.3%, polychaete worms, bivalves, terrestrial crustaceans, and trash (each 2.4% respectively) [Table 1]. The gastropods comprised three species: an endemic hydrobiid snail *Heleobops bermudensis*, the Red-rimmed Melania (*Melanoides tuberculata*), and the Coffee Bean Snail (*Melampus coffeus*). *Heleobops bermudensis* occurred in 57.1% of all fecal samples and was obtained from terrapins captured in South Pond, Mangrove Lake, and North Pond. *Melanoides tuberculata* occurred in 35.7% of the fecal samples but was only obtained from

terrapins captured in South Pond, while *M. coffeus* only occurred in 4.8% of the fecal samples and was obtained from terrapins captured in Mangrove Lake.

The plant materials consisted mostly of mown grass fragments, saw-grass seeds, and green algae. None of the plant material appeared to have been digested and may have been ingested incidentally with animal prey (cf. Erasmus 2012). The terrestrial arthropods consisted of honey bees (*Apis mellifera*) (4.8% of the samples), small beetles (*Berosus infuscatus*), an isopod (*Armadillidium vulgare*), a millipede (*Julus* sp.), a big-headed ant (*Pheidole megacephala*), and an unidentified caterpillar (each represented in 2.4% of the samples). Vertebrate animal bones came from aquatic species and included fish from the family Cyprinodontidae—which occurred in 11.9% of the samples; an amphibian (the toad *Rhinella* [syn *Bufo*] *marinus*)—which occurred in 4.8% of the samples; and another terrapin (*Malaclemys terrapin*), probably scavenged—which occurred in 2.4% of the samples. The fecal samples containing arthropods and fish and vertebrate animal bones were acquired from terrapins captured in a variety of ponds. The samples that contained the burrowing polychaete worm (*Arenicola cristata*) and shell fragments from the Flat Mangrove Oyster (*Isognomon alatus*) all came from terrapins captured in Mangrove Lake. The single sample that contained a cigarette filter was obtained from a terrapin captured in South Pond. It is worth noting that most of the samples ($n = 33$ or 78.6%) that contained sediment also contained other dietary items, whereas nine samples

Table 2. Dry mass summary of all animal food items obtained from 33 fecal samples of Diamondback Terrapins collected from four sites combined (South Pond, Mangrove Lake, Trott’s Pond, and North Pond).

	<i>Melanoides</i>	<i>Heleobops</i>	<i>Melampus</i>	<i>Isognomon</i>	Insect	<i>Fundulus</i> bone	<i>Rhinella</i> bone	<i>Malaclemys</i> bone	<i>Polychaete</i>	TOTAL
	dry mass (g)	dry mass (g)	dry mass (g)	dry mass (g)	dry mass (g)	dry mass (g)	dry mass (g)	dry mass (g)	dry mass (g)	dry mass (g)
Proportion of	37.08	14.85	2.22	0.0595	0.117	0.139	1.17	0.0003	0.0153	55.65
total dry mass	66.6%	26.7%	3.99%	0.11%	0.21%	0.25%	2.1%	0.0005%	0.027%	100%

(21.4%) comprised only sediment. Female, male, and juvenile terrapins were all found to have ingested sediment, but none of the neonate terrapins produced feces that contained sediment.

Table 2 summarises the dry mass of all animal food items obtained from 33 terrapin fecal samples. It is evident that the three gastropod species made up most (97.3% of dry mass) of the collected material. Table 3 summarises their numbers and sizes. First, it can be seen that the terrapins ate very large numbers of *M. tuberculata* and *H. bermudensis*, and second that the gastropods were predominantly small in size (*M. tuberculata* mean SH 3.2 mm; *H. bermudensis* mean SH 1.7 mm). Thirdly, these data show that *H. bermudensis* had been consumed by all age classes (i.e., adults, juveniles, and neonates), whereas *M. tuberculata* had been consumed by adults and juveniles and the larger *M. coffeus* were found only in female adult samples. Most *H. bermudensis* measured <2 mm SH and *M. tuberculata* measured <3 mm SH. The majority (ca. 70%) of the *M. coffeus* snails ingested by the females measured 9–10 mm SH.

Further statistical analysis is compromised because a) many gastropod shells were broken, so unmeasureable, and b) there were not matched numbers of female, male, juvenile and neonate terrapins. However, it appears from Table 3 that adult females consumed rather larger prey than adult males. This is consistent with earlier studies of this markedly sexually-dimorphic species (Tucker et al. 1995).

Finally, it should be noted that the diet of neonate terrapins was extremely restricted (Table 1). Four out of five samples only contained remains of the gastropod *H. bermudensis*. The last sample also contained this species together with a little insect material. None of the neonate fecal samples contained sediment, presumably reflecting their terrestrial lifestyle.

Benthic Biotic Surveys within the Terrapins’ Wetland Environments

Pond Benthic Surveys

Only two species of aquatic gastropods were encountered during the Mangrove Lake surveys; the False Horn Shell (*Batillaria minima*) and *H. bermudensis*. Two species of aquatic gastropods were also encountered during the South Pond surveys; *H. bermudensis* and *M. tuberculata*.

Table 4 summarises the gastropod survey data for all three transects in both ponds. Gastropod abundance in Mangrove Lake varied along Transects 1 and 2. *Batillaria minima* and *H. bermudensis* were encountered in relatively low numbers at locations that comprised sediment only (*B. minima* range 0–28 snails m⁻², mean 3.0, SD 7.2, *n* = 52; *H. bermudensis* range 0–192 snails m⁻², mean 27.0, SD 47.7, *n* = 424); however abundance increased significantly at locations where widgeon grass (*Ruppia maritima*) was found (*B. minima* range 0–56 snails m⁻², mean 33.0, SD 27.8, *n* = 132; *H. bermudensis* range 252–772 snails m⁻², mean 474, SD 221.5, *n* = 1,896). Shell height of *H. bermudensis* along both transects ranged from 1–4 mm (mean 1.7 mm, SD 0.5, *n* = 580); *B. minima* ranged from 6.5–11 mm (mean 8.9, SD 1.0, *n* = 46). Pooling the data for each of the two separate transects in Mangrove Lake shows that *H. bermudensis* was more abundant than *B. minima* along the central axes of the pond.

All of the sample locations along Transect 3 in South Pond comprised sediment and both snail species were encountered in low numbers (*H. bermudensis* 0–4 snails m⁻², mean 0.4, SD 1.3, *n* = 4; and *M. tuberculata* 4–20 snails m⁻², mean 13.2, SD 5.7, *n* = 132). Shell heights of *H. bermudensis* encountered along Transect 3 all measured one mm and the shell heights of *M. tubercu-*

Table 3. Pooled summaries of the total numbers (*n*) and sizes (shell height, SH) for whole *Melanoides tuberculata*, *Heleobops bermudensis*, and *Melampus coffeus* obtained from the 28 Diamondback Terrapin fecal samples that contained gastropods.

Terrapin samples	<i>n</i>	<i>Melanoides tuberculata</i>			<i>n</i>	<i>Heleobops bermudensis</i>			<i>n</i>	<i>Melampus coffeus</i>		
		Size Range (SH; mm)	Mean (SH; mm)	SD (mm)		Size Range (SH; mm)	Mean (SH; mm)	SD (mm)		Size Range (SH; mm)	Mean (SH; mm)	SD (mm)
All pooled	2224	1–18	3.2	2.1	1910	1–5	1.7	0.7	13	7–11	9.4	1.1
Female pooled	2112	1–18	3.3	2.1	1643	1–5	1.8	0.8	13	7–11	9.4	1.1
Male pooled	99	1–7	2.1	1.0	150	1–3	1.5	0.6	-	-	-	-
Juvenile pooled	13	1–3	2	0.6	77	1–3	1.2	0.4	-	-	-	-
Neonate pooled	0	-	-	-	40	1–2	1.2	0.4	-	-	-	-

Table 4. Summary of gastropod abundance (number of snails 0.25 m⁻²) at each sampling site along Transects 1 and 2 in Mangrove Lake and Transect 3 in South Pond.

Site No.	Description	<i>Batillaria</i>	<i>Heleobops</i>	<i>Melanoides</i>
1-1	sediment	2	9	0
1-2	sediment	1	10	0
1-3	sediment	0	0	0
1-4	sediment	7	6	0
1-5	sediment	0	0	0
1-6	sediment	0	4	0
1-7	widgeon grass	14	123	0
1-8	widgeon grass	14	63	0
1-9	sediment	0	4	0
1-10	sediment	0	48	0
2-1	sediment	0	0	0
2-2	sediment	0	1	0
2-3	sediment	1	0	0
2-4	sediment	0	1	0
2-5	sediment	0	1	0
2-6	widgeon grass	0	193	0
2-7	widgeon grass	5	95	0
2-8	sediment	0	15	0
2-9	sediment	0	4	0
2-10	leaf litter	2	3	0
3-1	sediment	0	0	1
3-2	sediment	0	1	4
3-3	sediment	0	0	4
3-4	sediment	0	0	4
3-5	sediment	0	0	3
3-6	sediment	0	0	5
3-7	sediment	0	0	5
3-8	sediment	0	0	3
3-9	sediment	0	0	1
3-10	sediment	0	0	3

lata ranged from 1–11 mm (mean 3.1 mm, SD 2.0). The pooled data for Transect 3 shows that *M. tuberculata* was more abundant than *H. bermudensis* within the sediment of South Pond. Furthermore, *H. bermudensis* appeared to be more abundant within Mangrove Lake than in South Pond.

Further analyses of gastropod abundances along the three transects were attempted. The data were non-normal and variance was heterogenous whether the data were raw or square root transformed. The requirements of parametric statistics were therefore violated. Accordingly, a non-parametric approach was adopted. First, the abundances of *B. minima* were investigated. A Kruskal-Wallis test across the three transects showed that there were significant differences amongst the numbers of this species (Chi-Square = 7.885, df = 2, $p = 0.019$). Post-hoc tests using Mann-Whitney U tests were then conducted

Table 5. Summary of gastropod (*Batillaria minima*) and crustacean (*Alpheus armillatus*) total abundance (individ. m⁻²) at each quadrat site ($n = 4$) within Mangrove Lake.

Site No.	Description	<i>Batillaria minima</i>	<i>Alpheus armillatus</i>
A	Sand and gravel	2128	0
B	Rocks	2000	48
C	Rocks	3504	32
D	Rocks	6752	0

to compare Transect 1 with Transect 2, Transect 1 with Transect 3 and finally Transect 2 with Transect 3. This is not an ideal approach as there is an attendant risk of Type 1 error (i.e., incorrect rejection of a null hypothesis), but no better alternative is available. These post-hoc tests indicated that there were no significant differences in numbers of *B. minima* between Transects 1 and 2 (both from Mangrove Lake) (Mann-Whitney U = 36.50, Wilcoxon W = 91.50, $Z = -1.153$, $p = 0.315$). There were no significant differences in numbers of *B. minima* between Transects 1 and 3 (Mann-Whitney U = 33.00, Wilcoxon W = 88.00, $Z = -1.302$, $p = 0.218$), but there were significant differences between Transects 2 and 3 (Mann-Whitney U = 12.00, Wilcoxon W = 67.00, $Z = -2.954$, $p = 0.003$).

Second, the same approach was adopted for the abundances of *H. bermudensis*. A Kruskal-Wallis test across the three transects showed that there were significant differences amongst the abundances of this species (Chi-Square = 12.76, df = 2, $p = 0.002$). Post-hoc Mann-Whitney tests showed that abundances of *H. bermudensis* did not differ between Transects 1 and 2 (Mann-Whitney U = 39.00, Wilcoxon W = 94.00, $Z = -2.954$, $p = 0.436$), but did differ significantly between Transects 1 and 3 (Mann-Whitney U = 11.00, Wilcoxon W = 66.00, $Z = -3.229$, $p = 0.002$) and between Transects 2 and 3 (Mann-Whitney U = 12.50, Wilcoxon W = 67.50, $Z = -3.117$, $p = 0.003$). Overall these tests indicate that there is strong (but not conclusive) support for the abundance trends identified above.

Table 5 shows the results of the four replicate quadrat surveys that were performed in the sandy, rocky, and gravelly marginal areas of Mangrove Lake. Only one species of gastropod (*B. minima*) and one species of crustacean (the Snapping Shrimp, *Alpheus armillatus*) were encountered. The snails were found most often attached to the rocky substrate, whereas the shrimp were found either buried within the gravel or hidden beneath rocks. The density of *B. minima* ranged from 2,000–6,752 snails m⁻² (mean 3,596, SD 2,211.4) and their sizes ranged from 3.5–10 mm SH (mean 6.4); the density of *A. armillatus* ranged from 0–48 shrimp m⁻² (mean 20, SD 24) and their total lengths (TL) ranged from 10–19 mm (mean 15.6). These data suggest that the density of *B. minima* surveyed upon the rocky shoreline habitat (mean 3,596 snails m⁻²) was nearly 400 times more than the mean density of live

Table 6. Biotic summary of the quadrat surveys ($n = 16$) performed within the mangrove swamp around Mangrove Lake. *M.c.* = *Melampus coffeus*, *M.m.* = *Myosotella myosotis*, *L.c.* = *Laemodonta cunensis*, *M.o.* = *Microtralia occidentalis*, *P.m.* = *Pedipes mirabilis*, *Amp.* = Amphipod spp., *L.b.* = *Ligia baudiniana*, *A.e.* = *Armadilloniscus ellipticus*, *A.v.* = *Armadillidium vulgare*, *B.i.* = *Bersos infuscatus*, *Lep.* = Lepidopteran larvae, *Jul.* = *Julus* sp., *A.m.* = *Anisolabis maritima*, *Fun.* = *Fundulus* eggs, *Ara.* = Arachnid spp., *P* = Earthworm sp.

	Gastropods					Crustaceans				Insects				Fish	Other	
	<i>M.c.</i>	<i>M.m.</i>	<i>L.c.</i>	<i>M.o.</i>	<i>P.m.</i>	<i>Amp.</i>	<i>L.b.</i>	<i>A.e.</i>	<i>A.v.</i>	<i>B.i.</i>	<i>Lep.</i>	<i>Jul.</i>	<i>A.m.</i>	<i>Fun.</i>	<i>Ara.</i>	<i>P</i>
Mean density (indiv. m ⁻²)	282	53	5	3	3	371	4	197	8	4	1	17	10	313	9	9
Size range (mm)	2–15	1–6	1–3	6–7	2–3	-	-	-	-	-	-	-	-	-	-	-
Mean size (mm)	8.8	2.8	1.8	6.3	2.3	-	-	-	-	-	-	-	-	-	-	-
SD	3.2	1.2	0.8	0.6	0.6	-	-	-	-	-	-	-	-	-	-	-

B. minima found upon the sediment along the central axes of Mangrove Lake (9.2 snails m⁻²).

Mangrove Swamp Surveys

Table 6 summarises the various aquatic and terrestrial species discovered during the quadrat surveys ($n = 16$) performed within this environment. A total of five gastropod species were encountered; all were found within the detritus of the intertidal zone and some individuals of *M. coffeus* were also encountered attached to Red Mangrove prop roots, usually in clusters, immediately above the water line of the pond. *Melampus coffeus* were most frequently encountered. Density for this species ranged from 0–1,168 snails m⁻² (mean 282, SD 399.3, $n = 4,512$), and shell height ranged from 2–15 mm SH (mean 8.8, SD 3.2, $n = 4,512$). *Myosotella myosotis* was the second most frequently encountered gastropod, but only at one of the 16 locations. Sizes ranged from 1–6 mm SH (mean 2.8, SD 1.2, $n = 848$). *Laemodonta cubensis* was encountered in densities of 80 snails m⁻² and all occurred in one location. Sizes ranged from 1–3 mm SH (mean 1.8, SD 0.8). *Microtralia occidentalis* and *Pedipes mirabilis* were infrequently encountered. Sizes of the former ranged from 6–7 mm SH (mean 6.3, SD 0.6, $n = 48$), and the latter ranged from 2–3 mm SH (mean 2.3, SD 0.6, $n = 48$).

In addition to the gastropods mentioned above, four species of crustaceans were encountered among the detritus (Table 5). The amphipods were the most abundant crustaceans encountered, being found in 81.3% of the quadrat locations. Densities ranged from 0–2,272 m⁻² (mean 371, SD 656.8, $n = 5,936$). The isopod *Armadilloniscus ellipticus* was the second most frequently encountered crustacean, with densities of 0–1,008 m⁻² (mean 197, SD 311.5, $n = 3,152$). *Ligia baudiniana* and *A. vulgare* were not commonly encountered.

Eggs (approx. two mm diameter) from the endemic Bermuda Killifish (*Fundulus bermudae*) were encountered in 25% of the quadrat surveys. Abundance varied from 0–3,824 eggs m⁻² (mean 313, SD 958.5, $n = 5,008$). The eggs were usually found hidden within the leaf detritus, but also attached to the Red Mangrove prop

roots at the high water mark. A variety of primarily terrestrial organisms were occasionally encountered in low densities within the 16 quadrat locations; these included millipedes, earwigs, small spiders, earthworms, small beetles, and a lepidopteran larva.

Saw-grass Marsh Surveys

Table 7 summarizes the aquatic and terrestrial species discovered during the quadrat surveys performed within this environment. Only one species of gastropod was found during the quadrat surveys (*H. bermudensis*). Densities ranged from 176–272 snails m⁻² (mean 208, SD 43.3, $n = 832$), and shell heights ranged from 1–4 mm SH (mean 2.3, SD 0.7). Terrestrial organisms were infrequently encountered within the quadrats and consisted of millipedes and small spiders. The number of saw-grass shoot bundles ranged from 16–48 m⁻².

Discussion

The anchialine ponds inhabited by Bermudian Diamond-back Terrapins are unusual habitats for the species. In the USA terrapins live predominantly in *Spartina* salt marshes and in the Everglades mangrove swamps of west Florida. The latter environments feature substantial allochthonous inputs from neighbouring marine and freshwater habitats as well as abundant autochthonous energy sources, so are amongst the most productive natural environments in the world, supporting diverse plant and animal communities (Schmalzer 1995; Whitney et al. 2004).

In contrast, energy sources of anchialine pools are

Table 7. Biotic summary of the quadrat surveys ($n = 4$) performed within the saw-grass marsh habitat at the center of South Pond. Note: results standardized to values m⁻².

Site No.	No. of grass shoot bundles	<i>Heleobops bermudensis</i>	Millipedes	Spiders
Q1	16	176	48	64
Q2	48	272	32	80
Q3	32	192	64	48
Q4	32	192	16	32

largely autochthonous. The Bermudian anchialine pools inhabited by terrapins proved to have limited faunal diversity. Over most of the area of Mangrove Lake (the largest pond), only two species of benthic gastropod snails were found; *H. bermudensis* and *B. minima*. Similarly, two species of aquatic gastropods were encountered during the benthic South Pond surveys; *H. bermudensis* and *M. tuberculata*. All three species are operculate deposit-feeders; *B. minima* and *H. bermudensis* are native, while *M. tuberculata* is primarily a freshwater (though salt-tolerant) species that is native to tropical and sub-tropical regions of southern Asia and northern Africa (Clench 1969), but widely-introduced to various regions via the aquarium trade. *Heleobops bermudensis* is a small endemic hydrobiid snail, limited to brackish-water ponds in Bermuda (see Pilsbry in Vanatta 1911), while *B. minima* is found also on local mudflats (Sterrer 1986).

The results of the quadrat and transect surveys revealed that the sediment surface in Mangrove Lake and South Pond generally showed relatively low densities of gastropods; however *B. minima* and *H. bermudensis* were both found to exist in higher densities in localized patches throughout Mangrove Lake. *Batillaria minima* was most often associated with sand, rock, and gravel substrate, reaching densities ca. 6,750 snails m⁻², whereas *H. bermudensis* was more commonly found within beds of widgeon grass in densities up to 772 snails m⁻². Benthic mapping of Mangrove Lake was not performed, but visual assessments of the pond in 2011 suggested that both the gravel/rock and widgeon grass environments comprised a very small proportion (< 5%) of the total pond area. Taken with the fecal sample results, it would appear that juvenile and adult terrapins on Bermuda rely heavily on benthic dredging of small gastropods (Outerbridge and Davenport 2013) from the large areas of pool bottoms, presumably because this unselective feeding behavior provides them with plenty of food.

Gastropods were more abundant and diverse within the mangrove and saw-grass marsh environments. Five species of gastropods (all pulmonates of the Family Melampidae) were encountered during the quadrat surveys within the detritus of the mangrove swamp intertidal zone around Mangrove Lake. *Melampus coffeus* grow to 20 mm SH, but the other species rarely exceed eight mm SH (Sterrer 1986). Thomas et al. (1992) and Herjanto (1994) reported that *M. coffeus* was frequently encountered upon the detritus and prop roots of mangrove trees in Mangrove Lake and Trott's Pond. The present investigation showed that gastropods within Bermuda's saw-grass marsh and mangrove swamp environments can reach densities of up to 1,168 snails m⁻² (*M. coffeus*). However, it is evident that the adult and juvenile terrapins rarely, if ever, use this resource and are essentially aquatic foragers.

Crustaceans were rarely encountered within the

aquatic environment of Mangrove Lake; only one species (*Alpheus armillatus*) was found in the rocky marginal habitats; no crustaceans were encountered within South Pond. However, crustaceans (mostly small amphipods and isopods) were frequently encountered (87.5%) in the quadrat surveys performed in the mangrove swamp surrounding Mangrove Lake. The Mangrove Crab (*Goniopsis cruentata*) was not encountered during the present study though it was reported to inhabit the intertidal zone of Mangrove Lake and Trott's Pond two decades ago (Thomas et al. 1992). Small numbers of terrestrial invertebrates were also found in the vegetated areas around the pools.

Some potential food organisms had been surveyed before this study. The Flat Mangrove Oyster (*Isognomon alatus*) grows in clumps on the submerged prop roots of red mangrove trees in Mangrove Lake and Trott's Pond and has been reported to reach densities of 250 oysters root⁻¹ or about 2,700 oysters m⁻² of pond (Thomas and Dangeubun 1994); the Bermudian terrapins hardly use this resource. Fish have also been investigated; the endemic killifish (*Fundulus bermudae*) occurs in Mangrove Lake (estimated population about 11,000) and Trott's Pond (about 8,000) (Outerbridge et al. 2007). Killifish in Mangrove Lake are benthopelagic and are omnivorous opportunistic feeders. They are swift swimmers that form loose schools of similarly-sized fish (Rand 1981) and are probably difficult for terrapins to catch.

Overall, it appeared that the ponds themselves had low faunal diversities, but abundant supplies of small deposit-feeding gastropod snails; the neighbouring vegetated areas had rather higher diversities, but gastropods were again dominant. Given the small size of the terrapin population (ca. 100 individuals \geq 81 mm straight carapace length, see Outerbridge et al., In Press), it was evident that plenty of food was available to them.

The benthic sediment in all of the terrapin ponds is gelatinous and extremely flocculent which allows the terrapins to both easily move through it and process it, apparently allowing them to consume *M. tuberculata*, the most frequently encountered gastropod within the pond's sediment (Outerbridge and Davenport 2013). In support of this hypothesis, fecal analyses from this study confirm that Bermuda's terrapins consume very high numbers of small (<2 mm) *M. tuberculata* and *H. bermudensis* together with large quantities of sediment. The sediment is believed to have been incidentally rather than deliberately ingested (as is probably the case for plant material too). It is evident that small gastropods form almost all of the adult and juvenile terrapins' animal diet (97.3% of dry mass).

All of the few insects recorded from fecal material were probably consumed after falling into the ponds, rather than having been ingested in the terrestrial environment (with the exception of those consumed by neonate terrapins which are residents of the intertidal mangrove and grass-dominated marsh environments

adjacent to the ponds; Outerbridge 2014). The fish, toad, and terrapin bones discovered in some fecal samples indicate that Bermuda's terrapins also scavenge on animal remains. Carcasses of these species are periodically observed floating at the surface of the study ponds and it is likely that they are opportunistically ingested when encountered. Scavenging has been reported for other diamondback terrapin populations in the USA (Ehret and Werner 2004; Petrochic 2009; Butler et al. 2012).

Plant material (mown grass fragments, saw-grass seeds, algae) was found in small quantities in a third of fecal samples. All appear to have been incidentally ingested. Mown grass fragments presumably reflect the golf course management of the terrapins' habitat. The presence of seeds in feces has been reported before (Tulipani 2013; Tulipani and Lipcius 2014) from terrapins foraging in salt marshes in Virginia; in that case the turtles were shown to be significant in the dispersal of Eelgrass (*Zostera marina*) seeds.

It is interesting to note that Bermuda's Diamondback Terrapins apparently did not ingest or rarely ate some items common in their environment. There was no evidence that they ever ate the Snapping Shrimp *Alpheus armillatus*, though substantial numbers were available in rocky areas of the shoreline. They also ate few of the Mangrove Oysters (*Isognomon alatus*) despite the latter's high population densities on mangrove roots. There was little evidence of foraging amongst the mangrove vegetation; most of the pulmonate gastropod species (*M. coffeus* does not appear to be an important dietary food item for Bermuda's terrapins, and *M. myosotis*, *L. cubensis*, *M. occidentalis*, and *P. mirabilis* do not appear to be consumed at all), amphipods and isopods were not recorded in fecal samples.

The dietary specialization and restriction in Bermuda's terrapins carries penalties. It has been demonstrated that they are exposed to a wide range of toxic compounds (e.g., trace metals, gasoline-range, and diesel-range petroleum hydrocarbons and polycyclic aromatic hydrocarbons) via food-chain contamination, specifically through the ingestion of gastropods, but probably exacerbated by the high incidence of associated sediment intake. It has also been shown that these contaminants are transferred to terrapins eggs, which show low hatching rates and evidence of embryonic abnormalities (Outerbridge et al. 2016).

Conclusion

The field surveys and fecal analyses reported on here showed that Diamondback Terrapins in Bermuda are specialist microphagous molluscivores that do not exploit the full range of potential prey species available to them. The range of food items ingested is much narrower than reported from North American populations, but this is probably caused by the near absence of tidal action that permits the accumulation of organic-rich

sediments browsed upon by abundant small gastropods. The anchialine pools and surrounding vegetated areas exhibit a low potential prey diversity in comparison with those found in the salt marshes of the eastern seaboard of the USA, but adult and juvenile terrapins evidently select preferentially within this low diversity for small gastropods of only two species (*M. tuberculata* and *H. bermudensis*).

Acknowledgments.—We are grateful to the Mid Ocean Club for granting access to the study site and wish to express our thanks to S. Massey, M. Hoder, P. Harris, and E. Limerick for their invaluable assistance with field work. Funding for this study was provided by the Atlantic Conservation Partnership, the Bermuda Zoological Society, and the Mid Ocean golf club. This is contribution #243 of the Bermuda Biodiversity Project (BBP) Bermuda Aquarium, Natural History Museum and Zoo, Department of Environment and Natural Resources.

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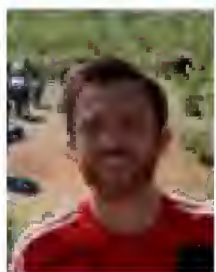
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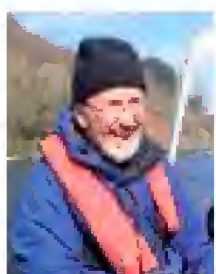
Mark Outerbridge works for the Bermuda Government at the Department of Environment and Natural Resources and received his Ph.D. from the University College Cork (Ireland). He has spent the last decade studying a wide variety of threatened and endangered species on Bermuda and also has a professional interest in the impact that invasive, non-native species have upon Bermuda's fragile island-ecosystems.



Ruth O'Riordan is the head of the Graduate School and a senior lecturer at the School of Biological, Earth and Environmental Sciences at the University Cork College (Ireland). Her research focuses on temperate and tropical intertidal ecology, supply-side ecology of marine invertebrates, biology and ecology of exotic aquatic species, climate change, and behavior of vertebrate animals.



Thomas Quirke is currently a lecturer within the Animal Management Department at Reaseheath College in the United Kingdom. After completing a B.S. in Zoology at University College Cork in Ireland, Thomas then moved on to complete his Ph.D., studying cheetahs within zoos in Ireland, the UK, Canada, and Southern Africa. He next spent a year at the University of Pretoria and the National Zoological Gardens of South Africa studying how animal personality influences the effects of environmental enrichment.



John Davenport is Emeritus Professor of Zoology at University College Cork (Ireland) and holds a D.Sc. from the University of London. A professional marine biologist since the 1970s, he has collaborated with Bermudian scientists since the 1980s, working on fish, skinks, and turtles.



Conservation status of Amphibians of Argentina: An update and evaluation of national assessments

^{1,3}Marcos Vaira, ¹Laura C. Pereyra, ¹Mauricio S. Akmentins, and ²Jon Bielby

¹Instituto de Ecorregiones Andinas (INECOA), CONICET, Universidad Nacional de Jujuy, Av. Bolivia 1711 (4600), San Salvador de Jujuy, ARGENTINA ²Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UNITED KINGDOM

Abstract.—We present a review on the conservation status of the 177 species and subspecies of amphibians of Argentina and compare the first national assessment, conducted in 2000, with the most recent one, from 2012, to determine changes in conservation status over time. We also evaluate the degree of taxonomic and geographic non-randomness in extinction risk among these taxa. The present study shows an improvement in the knowledge of amphibian diversity in Argentina, but also increasing evidence of population declines and species absences. Twenty-two species showed a genuine increase in threat status between national assessments, and habitat loss and/or degradation, chytrid fungus infection, and introduction of invasive species have been reported as the main threats. Randomization tests showed families Telmatobiidae and Batrachylidae to be over-threatened and Hylidae and Leptodactylidae to be significantly under-threatened. Also, four ecoregions were shown to be significantly over-threatened (Patagonian Steepe, Patagonian Woodlands, Puna, and Yungas Forests). This evaluation help to identify groups of species that face similar suites and intensities of threat as a result of their overlapping geographical distributions and shared biological susceptibility as a result of their evolutionary history. We consider that our results highlight patterns and trends to alert policymakers and to guide priority actions.

Keywords. Batrachylidae, diversity, ecoregions, Hylidae, Leptodactylidae, threats

Citation: Vaira M, Pereyra LC, Akmentins MS, Bielby J. 2017. Conservation status of amphibians of Argentina: An update and evaluation of national assessments. *Amphibian & Reptile Conservation* 11(1) [General Section]: 36–44 (e135).

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Received: 26 April 2016; **Accepted:** 25 November 2016; **Published:** 31 January 2017

Introduction

The widespread loss of biological diversity on a global scale poses a challenge demanding effective methods to assess the threat status of the biodiversity at a range of spatial scales (Mace et al. 2008). Global, regional, and/or local assessments of the status of species according to their extinction risk are important tools for guiding the development of conservation planning policies and regulations. Since most conservation actions are based on the threat category assigned to the species, the implementation of more efficient public policies and the improvement of public awareness may depend on reliable species information and assessments (Hoffmann et al. 2010).

Argentina harbors the tenth largest amphibian fauna among the 40 countries included in the Neotropical Realm. The Argentine amphibian fauna is also highly endemic, being among the twenty countries in the world

in which 30% of its amphibian species are endemic (Bolaños et al. 2008; Lavilla and Heatwole 2010). Like in most Neotropical countries, there are major gaps of information on the amphibian species of Argentina including systematic, genetic, range size, natural history, and ecology (Lavilla and Heatwole 2010). The usual barriers faced to accurately assess the threat status of amphibians in such countries are the many remote or unexplored regions coupled with relatively few scientific experts to detect, identify, and study species and/or populations, and the limited resources available to evaluate them (Becker and Loyola 2008; Brito 2008). Although challenging, the sum of individual efforts by amphibian researchers allowed, the development of the first national Red List of amphibians of Argentina in 2000, using a locally designed categorization method (Lavilla et al. 2000). Other contributions later summarized and updated the information on Argentinean amphibian diversity,

Correspondence. ³marcos.vaira@gmail.com

geographic distribution, and description of the principal threats (Lavilla and Cei 2001; Lavilla et al. 2002; Lavilla and Heatwole 2010).

Given the substantial increase in the rate of amphibian species description (Köhler et al. 2008), continual updating of existing conservation assessments is necessary in order to properly maintain that assessment's value (Stuart 2007). A status review requires the compilation of new information from new species descriptions, new taxonomic arrangements, and new evidence or research, and in most cases it is necessary to reassess the consensus of several experts about conservation status (Lukey et al. 2010). To accomplish this task, a new assessment of the conservation status of the amphibians of Argentina was published in 2012. The updated assessment shows an improvement in the knowledge of amphibian diversity in Argentina, but also increasing evidence of population declines and species absences (Vaira et al. 2012).

Nevertheless, none of the existing assessments or updates on the diversity and conservation status of Argentinean amphibians evaluate whether phylogenetically related species or those sharing similar distributional ranges face similar kinds of threats. Taxonomic and geographic selectivity in threats has been observed in some vertebrates, consisting of non-random distributions of the extinction risk of species among families or regions (Russell et al. 1998). Species have different probabilities of extinction depending on intrinsic factors like body size, population size, and genetic variability (Sodhi et al. 2008). Moreover, the probability of extinction rely also on external factors such as human disturbance, disease, habitat loss, and other threatening processes, as well as on the interaction between such extrinsic and intrinsic factors (Bennett and Owens 1997), and even stochastic events associated with small population sizes (Schaffer 1981). It is therefore possible that species that share some of these factors will have similar levels of threats. Thus, an evaluation of taxonomic and geographic patterns in the threat status of amphibians of Argentina could be used to focus conservation practices on entire clades or particular biogeographical regions rather than on individual species (Mace et al. 2003; Bielby et al. 2006; Corey and Waite 2008).

Given the reported improvement in the knowledge of amphibian diversity in Argentina (Vaira et al. 2012), coupled with changes in the conservation status and the intensity of threatening processes, prompted us to analyze changes in species' conservation status between the first comprehensive Argentinean conservation assessment (Lavilla et al. 2000) and the most recent one (Vaira et al. 2012). We are interested to know if reported changes are attributable to genuine improvement or deterioration of their conservation status or attributable to improved knowledge on taxonomy, ecology or distribution of the species. We also evaluate the degree of non-random-

ness in threat status of the species by taxonomy and geographic distribution to analyze whether threat status was randomly distributed across taxonomic families or regions.

Materials and Methods

The method used to categorize threatened species in Argentina was originally proposed by Lavilla et al. (2000), adapted from the method of Reca et al. (1994). More recently, Giraudo et al. (2012) reviewed the method with the aim to improve consistency and provide guidance on the assessment process. The categories are: Insufficiently Known (IC), Not Threatened (NA), Vulnerable (VU), Threatened (AM), and Endangered (EP).

We evaluated the changes in the conservation categories of the different taxa between the two assessments. A status change due to reported increase or decrease of threats was considered a "genuine" change. Those changes attributable to improved knowledge of both geographic distribution and taxonomy of the taxa were considered as "non-genuine" status changes (adapted from Hoffmann et al. 2010).

We followed the analytical methods proposed by Bielby et al. (2006) to analyze whether threat status was randomly distributed across taxonomic families and regions. We combined the categories VU, AM, and EP as Threatened and retained category NA as Not-threatened. The taxa in the IC category were omitted from the analysis, in order to remove the effects of non-random lack of knowledge for conservation status (Bielby et al. 2006). We then constructed two data sets ordering threat categories by taxonomic family or region. We assigned taxa range distributions to regions following the ecoregion classification scheme described in Lavilla and Heatwole (2010).

For each of the data sets, we first conducted a chi-square test to test for deviation from the null expectation that threatened taxa are distributed randomly among families or ecoregions. When non-random extinction risk was detected, we conducted further analyses to determine which families or ecoregions deviated from the expected level of threat. We did this by using a binomial test to calculate the smallest family size necessary to detect a significant deviation from the observed proportion of threatened taxa and excluded the families represented by an insufficient number of taxa.

For taxa in the remaining families, we generated a null frequency distribution of the number of threatened species from 10,000 unconstrained randomizations, by randomly assigning the categories to all remaining taxa. We then compared the actual number of threatened taxa in the datasets with the null frequency distribution. The null hypothesis (extinction risk is taxonomically and geographically random) was rejected if this number fell in the 2.5% at either tail of the distribution.

Table 1. Taxonomic arrangements of amphibians of Argentina not included in the list of the 2012 national assessment by Vaira et al. (2012).

Taxon name listed in Vaira et al. 2012	Changed to	Source
<i>Alsodes gargola gargola</i>	<i>Alsodes gargola</i>	Blotto et al. 2013
<i>Alsodes gargola neuquensis</i>	<i>Alsodes neuquensis</i>	Blotto et al. 2013
Not listed	<i>Elachistocleis haroi</i>	Pereyra et al. 2013
Not listed	<i>Oreobates berdemenos</i>	Pereyra et al. 2014
<i>Pseudis limellus</i>	<i>Lysapsus limellum</i>	Garda et al. 2010
<i>Somuncuria somuncurensis</i>	<i>Pleurodema somuncurens</i>	Faivovich et al. 2012
<i>Leptodactylus diptyx</i>	<i>Adenomera diptyx</i>	Pyron and Wiens 2011

Results

Update and summary of the conservation status of the amphibians of Argentina

Based on taxonomic changes and the description of two new species since 2012, updated list of amphibians of Argentina consists of a total of 17 families, 42 genera, and 177 species and subspecies (Tables 1 and 2).

Twenty taxa registered a “genuine” status change in its threat categories when comparing the 2000 Argentinean conservation assessment with the 2012 list, (Table 2). Most status changes represent an increase in the threat categories for the taxa due to: population decline (65%), habitat deterioration (25% of taxa), invasive species (10% of taxa), or by infection caused by the chytrid fungus *Batrachochytrium dendrobatidis* (10% of taxa). (Table 3). Twenty-five taxa registered “non-genuine” status changes attributable to improved knowledge of taxonomy or geographic distribution, while 19 taxa maintained the same threat category as listed in the first national assessment (Table 2).

Degree of non-randomness in threats of the species

The family data set showed a significant deviation from a random distribution of threatened species and subspecies among the amphibian families ($\chi^2 = 76.5$, $df = 9$ $P < 0.001$). Randomization tests showed two families to be significantly overthreatened (Telmatobiidae and Batrachylidae with 100% and 60% of their taxa threatened, respectively) and two families to be significantly underthreatened (Hylidae and Leptodactylidae with 5% and 14% of the taxa threatened, respectively) (Tables 2 and 4).

The ecoregions data set also showed a significant deviation from a random distribution of threatened taxa ($\chi^2 = 140.25$, $df = 14$ $P < 0.001$). Randomization tests showed four ecoregions overthreatened (Fig. 1): Patagonian Steepe (75% of the taxa threatened); Patagonian Woodlands (65% of the taxa threatened); Puna (71% of the taxa threatened), and Yungas Forests (29% of the taxa threatened) [Table 5].

Discussion

This update shows a substantial improvement in the knowledge of amphibian diversity in Argentina since the first major assessment in 2000, with 11 new species described (see Vaira et al. 2012) and improved knowledge on taxonomy and/or geographic distribution of several species reflected by 16 taxa decreasing their threat categories and changing status as a consequence of the amount of information (i.e., the “non genuine” status change).

Unfortunately, there is also evidence of taxa increasing their threat status in Argentina since the first national conservation assessment. Habitat loss and/or degradation, chytrid fungus infection, and introduction of invasive species have been considered as principal threats suggested for eight species changing to higher threat categories (Vaira et al. 2012). Nonetheless, the lack of studies that simultaneously evaluate the importance of those threats on the species is notable.

Major concerns constitute the lack of registries verification of these four species in the wild for prolonged time lapses: *Telmatobius ceiorum*, *T. laticeps*, *Gastrotheca christiani*, and *G. chrysosticta* (Barrionuevo and Ponssa 2008; Akmentins et al. 2012) even after exhaustive surveys conducted in recent years within their natural geographic ranges. Whether these species are still extant is uncertain. Nevertheless, it is generally recommended to be extremely cautious to declare a species extinct because of the conservation implications involved, and it seems appropriate to encourage additional conservation efforts until there is no reasonable doubt of its extinction (Mace et al. 2008; Akmentins et al. 2012). However, to better reflect the likelihood of these species becoming extinct under prevailing circumstances we suggest that their global conservation status should be reconsidered.

Like in most Neotropical countries, there are major gaps of information on the amphibian species of Argentina including genetic, geographic range size, natural history, and ecology. The status of most of their populations is unknown since there is simply not enough information to estimate or infer a trend. Despite limited information, reported declines and identified threats require quick decisions on prioritizing conservation actions on certain

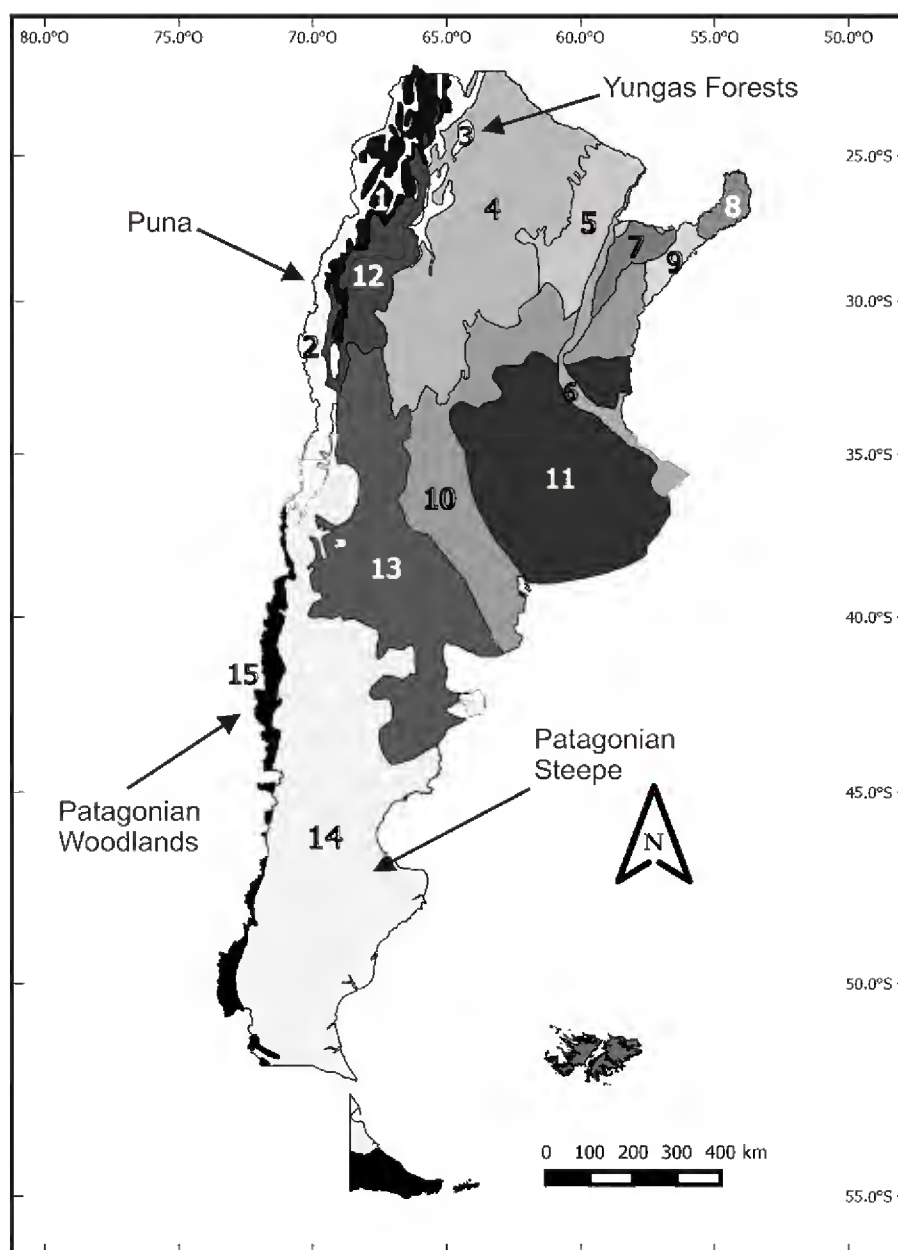


Fig. 1. Map of the ecoregions of Argentina from Burkart et al. 1999. Numbers indicate ecoregions divisions as follows: (1) Puna, (2) High Andean, (3) Yungas Forest, (4) Dry Chaco, (5) Humid Chaco, (6) Delta and Islands of the Parana River, (7) Esteros of Ibera, (8) Paranaen Forest, (9) Campos and Malezales, (10) Espinal, (11) Pampas, (12) Monte of Sierras and Bolsones, (13) Monte de Llanuras and Mesetas, (14) Patagonian Steepe, (15) Patagonian Woodlands. Arrows indicate the four overthreatened ecoregions.

species over others. We encourage the development and implementation of a conservation action plan of threatened amphibian species of Argentina through specialist consensus.

Hylidae and Leptodactylidae were found to be under-threatened families probably because they both contain taxa with large geographic distributions and small numbers of endemic species (Lavilla and Heatwole 2010). Randomization test results suggest that Telmatobiidae and Batrachylidae clades are overthreatened and hence may be especially prone to extinction. Reported threats within these families are consistent with the possibility that shared evolutionary history per se is an important precursor to vulnerability. The genus *Telmatobius* shares ecological traits present in many amphibians that have declined worldwide such as restricted distributions in high mountain ranges, low fecundity, and aquatic adults (Lips et al. 2003; Sodhi et al. 2008; Bielby et al. 2006). Then, endemic populations of *Telmatobius* should be more prone to extinction from environmental and demographic stochasticity which prompts us to consider how

severe the human impacts on those species will be. Four possible factors have been suggested as causes of decline for the species of *Telmatobius* in Argentina: unusual climate coupled to an increase in erosive processes and debris flowing events in montane streams, introduction of exotic predatory fishes in the river basins, and chytrid fungus infection (Barrionuevo and Ponsa 2008; Vaira et al. 2012). Similar results were reported in Ecuador, where the most critically endangered species belonging to *Telmatobius* genera occurred in regions characterized by drier conditions and high suitability for *Batrachochytrium dendrobatidis* (Menéndez-Guerrero and Graham 2013).

Species with similar life history traits and habitat use patterns are likely to be more sensitive to environmental instability and are less able to adapt to or recover from environmental or ecological changes (Sodhi et al. 2008). The evaluation of non-randomness of threat status can help to identify groups of related species that face similar suites of human-caused threat and biological susceptibility due to overlapping geographical distributions and shared evolutionary history respectively. This approach can help us to pinpoint needs for emergency action and to alert policymakers and conservation managers. This knowledge could be used to plan future protected areas where threat is concentrated and to guide mitigation measures. *Telmatobius* and *Atelognathus* might be examples of such an approach, setting conservation actions in the specific ecoregions where these genera inhabit (Patagonian Steepe and Woodland, Puna and Yungas Forests) and to manage their specific threats (e.g., introduction of predatory fishes and/or mining) that might yield better results than directing resources towards single species or individual populations.

On the other hand, broadly distributed species represent another challenge for setting conservation priorities if they comprise evolutionary lineages that may be under different levels of threat. An assessment at country level may lead to conflicting results in the threat category of a species complex and the possibility of over or underestimation of their conservation status. It is conceivable that as studies continue, species which we now consider widespread may indeed be local endemics. Additionally, since the effects of threats can be expected to vary spatially, especially in heterogeneous environments, better assessments are needed across ecoregions and thus fully accomplish the conservation assessment of amphibians in Argentina.

Because our knowledge to estimate the risk of extinction of amphibians of Argentina is still rather limited, the identification of most threatened regions may prove useful in cases where available data limit the certainty of the assessment outcomes. Our approach based on the evaluation of non-randomness of threat status may help to identify regions that are at greater risk and to capture the attention of researchers and policymakers. Thus, our rather broad results may be refined for more concerted

Conservation status of amphibians of Argentina

Table 2. Comparison of the 2000 and 2012 national assessments of threatened species of Argentina showing number of taxa with the same, increasing, or decreasing threat categories. A status change due to reported increase or decrease of threats was considered a “genuine” change. Those changes attributable to improved knowledge of both geographic distribution and taxonomy of the taxa were considered as “non-genuine” status changes (adapted from Hoffmann et al. 2010).

Families	Threatened species and subspecies on 2000 Red List	Threatened species and subspecies on 2012 Red List	Number taxa same threat category	Number taxa increasing threat categories	Number taxa decreasing threat categories	“Genuine” status change	“Non-genuine” status change
Siphonopidae (3)	2	0	0	0	2	0	2
Typhlonectidae (1)	1	0	0	0	1	0	1
Alsodidae (9)	2	5	1	4	0	4	0
Batrachylidae (15)	11	9	7	1	3	0	4
Brachycephalidae (1)	0	0	0	0	0	0	0
Bufoideae (30) ^b	9 ^a	7 ^{a,b}	4	2 ^a	2 ^a	1 ^a	3 ^a
Centrolenidae (1)	1	0	0	0	1	0	1
Ceratophryidae (6)	0	1	0	1	0	1	0
Craugastoridae (3) ^{b,c}	1	2 ^b	1	1	0	0	1
Hemiphractidae (3)	3	3	0	3	0	3	0
Hylidae (38)	6	2	2	0	4	0	4
Hylodidae (2)	1	0	0	0	1	0	1
Leptodactylidae (37)	5	5	3	2	0	1	1
Microhylidae (4) ^{b,c}	0	0	0	0	0	0	0
Odontophrynidae (8)	2	1	1	0	1	0	1
Rhinodermatidae (1)	1	1	0	1	0	1	0
Telmatobiidae (15)	10	15 ^b	0	14	1	9	6

Notes: Taxonomy follows Frost (2015). See Vaira et al. (2012) for the complete list of species and subspecies considered (see also text of this contribution to account for a few nomenclatural changes at genera and species level).

^a The threatened taxa in 2000 and 2012 are not the same. See Vaira et al. (2012).

^b Include new species described after 2000.

^c Include one new species described after 2012 and not evaluated.

studies focused on particular regions to expand the assessment goals not only to identify species at risk but also threats to ecological and evolutionary processes.

Conclusion

Applications of the national assessment: Challenges and future directions

Compiling a national threatened species lists helps to reveal information gaps and stimulate data collection focusing on species or areas where there may be needed conservation actions and where more research may be required (Gärdenfors 2001). We have now a substantial body of knowledge that can provide insights on the conservation status of amphibians of Argentina. A remaining task should be to objectively evaluate the uncertainties of the national assessment. The performance of the national assessment may be improved by testing and refining the accuracy of protocols and criteria to ensure future reassessments in an objective, comparable, and repeatable manner. Also, we must foster better linkages between national and global assessment efforts (de Grammont and Cuarón 2006).

Much is still unknown about potential threats in most species of amphibians of Argentina and many groups exhibit high levels of data deficiency doing status assessments unevenly detailed across species (Vaira et al. 2012). Due to the limited number of empirical data for most species, the national assessment can assist in the identification of groups of species that are more prone to future declines under common threats, due to their shared traits and geographic distributions, constituting an alternative approach to integrate this knowledge into the development of coordinated strategies for data collection or into proactive conservation programs. Data of “genuine” changes in the status of threatened species can then be used to measure progress of programs, and also be used to inspire development of national policies and legislation to protect species and particular regions they inhabit.

An exclusive focus on species-based approaches to conservation planning is controversial (Sætersdal and Gjerde 2011; Nicholson et al. 2013). As better data and methodologies become available, defining priority areas for conservation constitute a most desirable goal (Jenkins et al. 2013). Ideally, we must also address the complexity of natural ecosystems including phylogenetic, ecolog-

Table 3. Species of amphibians of Argentina that increase their threat categories between the 2000 and the 2012 national Red List assessment. Only species with “genuine changes” were considered (see text for explanation). Reasons of changes follow Vaira et al. (2012). Values in parentheses indicate status deteriorations from a lower to a higher category of threat.

Species	Threat category in Argentina	Reason of change
<i>Alsodes australis</i>	VU (-1)	invasive species
<i>Alsodes gargola</i>	VU (-1)	habitat deterioration
<i>Alsodes neuquensis</i>	AM (-1)	invasive species
<i>Alsodes pehuenche</i>	EP (-3)	habitat deterioration
<i>Rhinella achalensis</i>	AM (-1)	habitat deterioration / Bd*
<i>Ceratophrys ornata</i>	VU (-1)	habitat deterioration
<i>Gastrotheca christiani</i>	EP (-2)	population decline
<i>Gastrotheca chrysosticta</i>	EP (-2)	population decline
<i>Gastrotheca gracilis</i>	EP (-2)	population decline
<i>Pleurodema somuncurens</i>	EP (-1)	habitat deterioration
<i>Rhinoderma darwini</i>	AM (-1)	population decline
<i>Telmatobius ceiorum</i>	EP (-2)	population decline
<i>Telmatobius contrerasi</i>	AM (-2)	population decline
<i>Telmatobius hauthali</i>	AM (-1)	population decline
<i>Telmatobius laticeps</i>	EP (-2)	population decline
<i>Telmatobius oxycephalus</i>	AM (-1)	population decline
<i>Telmatobius pisanoi</i>	AM (-1)	population decline / Bd*
<i>Telmatobius schreiteri</i>	AM (-1)	population decline
<i>Telmatobius scroechii</i>	VU (-1)	population decline
<i>Telmatobius stephani</i>	AM (-1)	population decline

Categories: Vulnerable (VU), Threatened (AM), Endangered (EP).

* Bd: Infection caused by chytrid fungus, *Batrachochytrium dendrobatidis*.

Table 4. Results of the analysis of distribution of threatened species in the amphibian families of Argentina after the omission of Insufficiently Known (IC) species. The null hypothesis (threat status is taxonomically random) was rejected if *P* values were equal or less than 0.025% at either tail. Families under or overthreatened are bolded. NA: families represented by an insufficient number of species from analysis.

Families	Threatened taxa/ Total # of taxa*	>Expected threat-level <i>P</i> -value	<Expected threat-level <i>P</i> -value
Alsodidae	5/7	0.04	NA
Batrachylidae	9/11	0.001	1
Bufonidae	7/25	0.74	0.49
Ceratophryidae	1/6	0.89	NA
Hemiphractidae	3/3	0.03	NA
Hylidae	2/37	1	<0.001
Leptodactylidae	5/36	1	0.01
Microhylidae	0/3	1	NA
Odontophrynidae	1/7	0.93	NA
Telmatobiidae	15/15	<0.001	1

* IC species omitted.

ical, and evolutionary processes (Lindenmayer et al. 2007). National conservation assessment disaggregated by habitats, ecosystems, or ecoregions can thus provide a valuable base to support the design of priority areas requiring us to translate assessments from country to regional or local levels.

A common confusion introduced in some national conservation assessment applications is to consider conservation status and conservation priorities as equals when they are related but different processes. Conservation status alone should not necessarily determine conservation priorities (de Grammont and Cuarón 2006). Assigning species to a threat category in a conservation assessment should be an objectively scientific process to estimate the risk of extinction of a species. By contrast, setting conservation priorities determine which species should be protected and will often involve political as well as logistical considerations, so it is possible to establish different sets of species with conservation priorities in different regions within the country. Both components are essential for better policy-making and for more accurate scenarios for conservation and management.

Many national or regional conservation agencies interpret conservation assessments as a priority-setting tool for conservation action (Miller et al. 2007). Sometimes, there is a direct connection between conservation assessments and conservation policies, basing protective legislation or conservation actions directly on conservation categories. This can have undesired consequences, such as Data Deficient species being disregarded when allocating resources for conservation or protection. We must consider an increased communication and cooperation between researchers and policy-makers for generating and using national conservation assessments to effective conservation actions and legislation.

Acknowledgments.—This project was partially supported by a PICTO-UNJu grant # 153 and a Sector-UNJu grant # D-084. We specially thank two reviewers for providing insightful comments that greatly helped us to improve the manuscript.

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Table 5. Results of the analysis of distribution of threatened species in the ecoregions of Argentina after the omission of Insufficiently Known (IC) species. The null hypothesis (threat status is geographically random) was rejected if *P* values were equal or less than 0.025% at either tail. Ecoregions under or overthreatened are bolded. NA: ecoregions represented by an insufficient number of species from analysis.

Ecoregions ¹	Threatened taxa / Total no. of taxa*	>Expected threat-level <i>P</i> -value	<Expected threat-level <i>P</i> -value
Campos and Malezales	1/41	0.997	0.169
Delta and Islands of the Paraná river	0/42	1	0.037
Dry Chaco	5/54	0.879	0.762
Espinal	2/40	0.978	0.392
Esteros of Iberá	1/44	0.998	0.137
High Andean	2/55	0.143	NA
Humid Chaco	2/51	0.995	0.228
Monte de Sierra and Bolsones	1/11	0.806	NA
Monte of Llanuras and Mesetas	2/9	0.352	NA
Pampan	5/26	0.279	0.986
Paranaean Forest	3/52	0.981	NA
Patagonian Steepe	6/8	<0.001	NA
Patagonian Woodlands	11/17	<0.001	NA
Puna	10/14	<0.001	NA
Yungas Forests	11/38	0.009	1

¹From Lavilla and Heatwole (2010); *IC omitted.

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Conservation status of amphibians of Argentina



Marcos Vaira is a researcher at the Instituto de Ecorregiones Andinas (CONICET – Universidad Nacional de Jujuy). His primary area of interest is amphibian diversity. The aim of our research is to contribute to a better understanding on the structure and functioning of amphibian communities in the subtropical montane forest landscapes of Northwestern Argentina to provide a solid framework for their conservation.



Laura Cecilia Pereyra is a researcher at the Instituto de Ecorregiones Andinas (CONICET – Universidad Nacional de Jujuy). Her primary area of interest is the study of multifaceted components of diversity combining measures to assess and compare amphibian diversity in human-modified forest landscapes of Northwestern Argentina.



Mauricio Sebastián Akmentins is a researcher at the Instituto de Ecorregiones Andinas (CONICET – Universidad Nacional de Jujuy). His primary area of interest is the ecology and conservation of direct-developing frogs of the subtropical montane forest of Northwestern Argentina.



Jon Bielby is a Research Fellow at the Institute of Zoology, London. He researches wildlife disease, population decline, and extinction risk, with a particular focus on amphibians.



Preliminary observations on the circadian variation in site fidelity in *Atelopus hoogmoedi* (Lescure, 1974) (Anura, Bufonidae)

¹Michaël P.J. Nicolai, ^{1,2}Sara Porchetta, ^{1,3}Shashank Balakrishna,
^{1,4}David P. Botha, and ^{1,5}Philippe J.R. Kok

¹Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, BELGIUM

Key words. Anura, *Atelopus hoogmoedi*, Guyana, homing behavior, Iwokrama, site fidelity

Citation: Nicolai MPJ, Porchetta S, Balakrishna S, Botha DP, Kok PJR. 2017. Preliminary observations on the circadian variation in site fidelity in *Atelopus hoogmoedi* (Lescure, 1974) (Anura, Bufonidae). *Amphibian & Reptile Conservation* 11(1) [General Section]: 45–50 (e136).

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Received: 25 February 2016; **Accepted:** 08 June 2016; **Published:** 31 January 2017

The genus *Atelopus* (Bufonidae) is mostly composed of conspicuously colored species, several of which are known to secrete toxins (Fuhrman 1969; Yotsu-Yamashita and Tateki 2010). The genus has recently received increased attention due to severe decline in population numbers, often suggested to have revealed extinctions (La Marca et al. 2005; Pounds et al. 2006; Wake and Vredenburg 2008; but see Luger et al. 2008 for the Guianas). This mass decline has been attributed to multiple factors such as habitat loss, pollution, introduced species, and the chytrid fungus *Batrachochytrium dendrobatidis* (La Marca et al. 2005). One of the most widespread, and probably less threatened species in the genus is *Atelopus hoogmoedi* (Fig. 1 A–B), which is found in French Guiana, Suriname, Guyana, and northern Brazil (Noonan and Gauthier 2005; Kok and Kalamandeen 2008; Luger et al. 2008; Segalla et al. 2014). Two color morphs co-occur syntopically in Iwokrama (Guyana), an orange and a yellow morph (Fig. 1 A–B). This diurnal toad exhibits spatio-temporal segregation of sexes, with males usually found near streams, while females are found deeper in the forest, away from water bodies (Luger et al. 2009). During the breeding season, which mostly occurs in the dry season (see below), females migrate to streams for mating (Fig. 1 C, Luger et al. 2009). Similar reproductive strategies are observed in other anurans and are often characterized by site fidelity in which the males remain in the vicinity of the same perching site for the duration of the breeding period (e.g., Roithmair 1992; Ringler et al. 2009).

Several studies have investigated homing behavior and site fidelity in some *Atelopus* species (e.g., Crump 1986), including *A. hoogmoedi* (Luger et al. 2009). It has been suggested that site fidelity increases the probability of finding a suitable mate by improving the detection either by males through an increased field of vision, or by females as a result of a more conspicuous male perch (Himmel 2013). However, the occurrence of site fidelity outside of the reproductive season makes the mate detection hypothesis unlikely (Crump 1986; Luger et al. 2009). Alternatively, site fidelity may provide better knowledge of the local microenvironment making foraging more efficient, as well as providing means of protection from predation e.g., by good knowledge on possible escape routes (Luger et al. 2009). Previous studies have focused on diurnal site fidelity, and investigations pertaining to circadian variation in site fidelity are lacking. Since protection from predation is a plausible hypothesis explaining site fidelity, nocturnal behavior (when the diurnal animal is most vulnerable and an optimal protective perch likely required) deserves further attention. If protection is indeed a major driver for site fidelity, scarcity of optimal refuges might lead to increased site fidelity.

This hypothesis was briefly tested during fieldwork conducted in the Iwokrama Forest Reserve, central Guyana. Iwokrama is mostly covered by tropical moist lowland forest (Holdridge 1967), with some of the Iwokrama Mountains reaching ca. 900 m asl (MPFITRF 2009). Climate in Iwokrama is tropical, with an annual mean temperature of 25 °C and a mean rainfall of 3,000 mm

Correspondence. ¹michaelnicolai22@hotmail.com (Corresponding author); ²saraporchetta@hotmail.com; ³rb.shashank@gmail.com; ⁴slangseun@gmail.com; ⁵Philippe.Kok@vub.ac.be (Corresponding author).

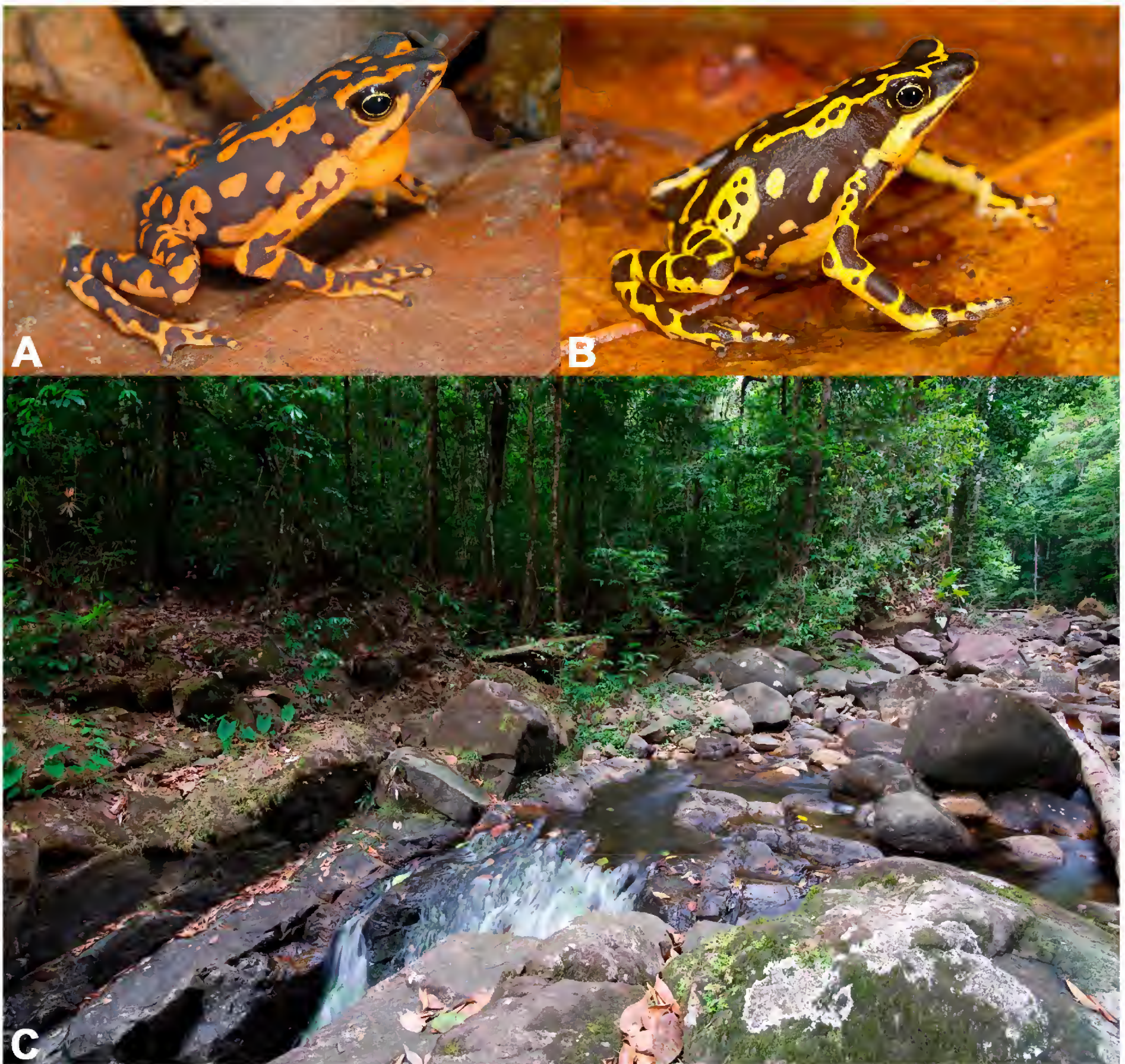


Fig. 1. (A) Orange and (B) Yellow color morphs of *Atelopus hoogmoedi*, both encountered at the studied locality in the Iwokrama Mountains, Guyana. (C) Typical breeding habitat of *A. hoogmoedi* in the Iwokrama Mountains. Photos by PJRK.

in the north of the reserve and 1,400 mm in the south of the reserve (MPFITRF 2009). Wet season usually extends from May to August and again from November to February (MPFITRF 2009), although this has seemed more irregular in recent years, especially during El Niño events (reported as particularly strong in 2015, and the months of November and December 2015 were very dry in Iwokrama).

Fourteen *Atelopus hoogmoedi* males were “marked” (see below) and “recaptured” in order to track their circadian variation in site fidelity. All individuals were spotted along a portion of the trail between Turu Falls and the Linden-Lethem road (between N 4°24.74' W 58°47.13' and N 4°24.63' W 58°47.30'; WGS 84; Fig. 2). Elevation ranged between 92 and 120 m asl. The trail was walked twice a day, once at 13h (daylight) and once at

18h (shortly after nightfall) between November 27 and December 1, 2015. Color pattern is individual-specific in this species (see Fig. 1 A–B), and individuals were identified based on photographs of the dorsal pattern taken in situ using a digital camera (Canon® Eos 7D). To limit physical interaction, ventral patterns were not examined (no specimen was manipulated during this study). The use of color pattern for identification in this species is an appropriate alternative to invasive marking techniques such as toeclipping (Luger et al. 2009). Locations of observations were recorded using a Garmin eTrex 30® GPS, and marked with colored flagging tape at the site of first detection, allowing immediate recognition of the site. For each observation three parameters were recorded: (1) height above ground; (2) perch type (categorized as leaf litter, shrub, or rock); and (3) distance from

original diurnal or nocturnal perch site. Distances were measured using a 50-m measuring tape. In order to standardize the procedure, an individual was considered to be present if three investigators were able to track it within a three-meter radius from the original perch site within three minutes. We used independent *t*-tests to compare the observation probabilities between different days and different sampling times. Additionally, Fisher's exact test was used to determine whether any of the two most common substrates (shrub or leaf litter) was used more than the other. All statistical analyses were done using IBM SPSS® v20.0.0.

Observation probabilities did not differ significantly between days ($t = 1.14$, $P = 0.26$), nor did they differ significantly between daylight (62%) and night (69%) ($t = 0.71$, $P = 0.54$). More individuals did, however, return to the original perch site at night than during the day (distance from original perch site = 0; $P = 0.08$). Finally, a difference in perching substrate use was found at different times of the day (Fig. 3). While this difference between substrate use was not significant during daylight ($P = 0.25$), a significant difference was found at night when most frogs were present on shrubs ($P =$

0.00). Unlike Luger et al. (2009), who detected *A. hoogmoedi* in leaf litter only 14% of the time, in our study leaf litter was the most used microhabitat, especially during daylight (64%). After dark most individuals were found perching in shrubs, at heights between 10–130 cm.

Most *Atelopus* species, including *A. hoogmoedi*, mate close to streams, and occurrences of flashfloods have been reported to wash away entire *Atelopus* populations (Duellman and Trueb 1986). As a result, high perching sites were explained as a strategy against such sudden water rises (e.g., Duellman and Trueb 1986; Luger et al. 2009). This could explain the differences in observations between our study and previous studies (Luger et al. 2009). Our study, in which individuals were often found on the ground, was conducted during the dry season (see above), in contrast with previous studies that were conducted during the wet season (protection from sudden water rises is likely more necessary during the latter). Furthermore, as reproductive interactions occur in the leaf litter (observation of amplexus during our fieldwork), male individuals are more likely to perch on the leaf litter during breeding season to increase the chance of inter-sexual interactions. Alternatively, differences

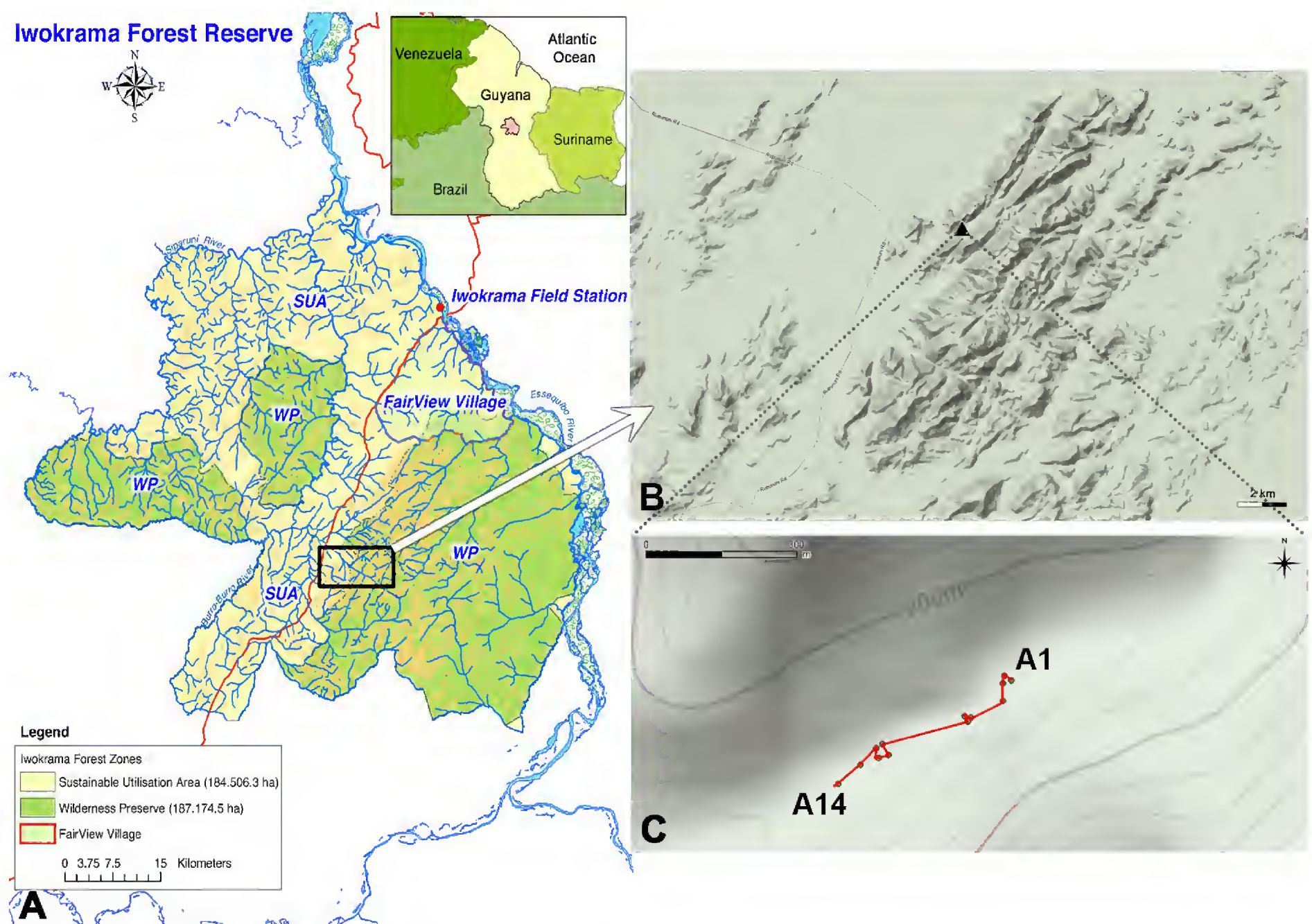


Fig. 2. Geographical overview of the study area. (A) Map of the Iwokrama Forest Reserve and its location in Guyana (top right corner). The red line crossing Iwokrama corresponds to the Linden-Lethem Road. (B) Relief map of the Iwokrama Mountains with Turu Falls represented by a black triangle (N 4°24.770' W 58°47.061'). (C) Portion of the trail between Turu Falls camp and the Linden-Lethem Road monitored, with dots corresponding to *Atelopus* individuals (from A1 - N 4°24.742', W 58°47.130' to A14 - N 4°24.750', W 58°47.128'). A and B from Kok et al. (2013).

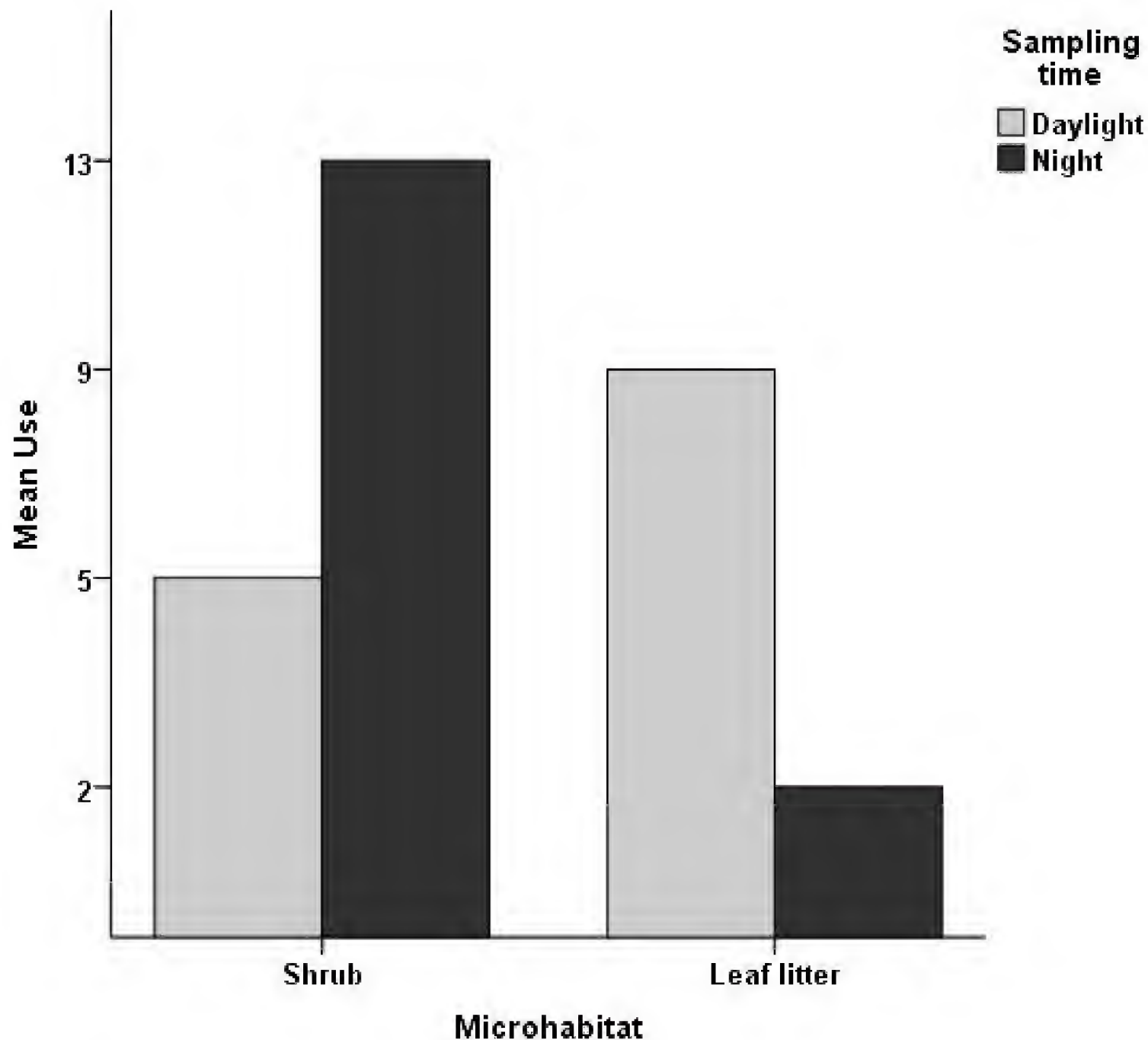


Fig. 3. Substrate (shrub or leaf litter) use in *Atelopus hoogmoedi* during the day (light grey) and night (dark grey) at Turu Falls, Guyana. As indicated, substrate use was significantly different after dark.

with Luger et al. (2009) might be explained by different abiotic and biotic factors between the two study sites. As previous studies indicated that perching on shrubs occurred during both breeding and non-breeding season (Crump 1986; Luger et al. 2009), it is unlikely that this elevated perching plays a major role in mating. The fact that the frogs leave these perch sites diurnally, when they are actively reproducing, further corroborates the hypothesis that elevated perching is not reproduction related. In other *Atelopus* species, such as *A. zeteki*, higher nocturnal perches are proposed to be a safe retreat from predators for this diurnal frog, shifting vigilance from visual to tactile (Lindquist et al. 2007). Some perches serve as better retreats than other perches, and difference in perch quality could drive both diurnal and nocturnal site fidelity. Our preliminary data indeed show that there was no significant difference in site fidelity between night and day as would be expected when site fidelity is linked to lower predation pressure. Furthermore, individuals returned more to the original nocturnal perching site than to the original diurnal perching site. This indicates that site fidelity might actually be linked to nocturnal perching site rather than to diurnal perching site. As both foraging and breeding occur during the day, the protection

hypothesis provides a good explanation for this nocturnal site fidelity, and site fidelity in general.

Some species of *Atelopus* are known to have lived over ten years in the wild (La Marca 1984), and at least one individual was recorded on the same boulder two years after the previous observation (Crump 1986). Such life history strategies make *Atelopus* ideal organisms for study of long-term site fidelity. Future studies are encouraged to expand our preliminary findings by increasing the length of the study, and if possible the number of recaptures.

In conclusion our observations, although sparse, seem to confirm that *Atelopus hoogmoedi* does indeed show strong diurnal and nocturnal site fidelity, during breeding and non-breeding seasons. Although several hypotheses may explain this, the fact that perch site return rate is the highest after dark supports the predation evasion hypothesis.

Acknowledgments.—These observations were made in the framework of the Field Herpetology course in Guyana provided to the second year students of the Master in Herpetology at Vrije Universiteit Brussel. We are indebted to Raquel Thomas (Iwokrama, Guyana) for grant-

ing us the permission to have this training course taught in the Iwokrama Mountains. We also thank Marcelo Kokubum (Universidade Federal de Campina Grande, Brazil), Stefan Lötters (Universität Trier, Germany), and Ross MacCulloch (Royal Ontario Museum, Canada) for comments that improved our original manuscript, and Ruben D.E. Culqui, Raheleh Dezfoulan, Yousri El Adak, and Bérengère Ferrier for help and companionship during fieldwork.

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Michaël Nicolaï is a Ph.D. student in the Amphibian Evolution Lab, Vrije Universiteit Brussel. He received his B.S. and M.S. in Biology at the KU Leuven (Belgium) as well as an additional master in Herpetology at the Vrije Universiteit Brussel. His main research interests are amphibian evolution, in particular the evolution of coloration in different ecomorphs of frogs.



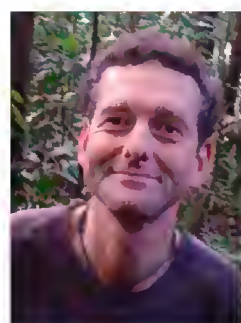
Sara Porchetta is a Ph.D. student at the Environmental and Applied Fluid Dynamics department of the von Karman Institute, Belgium. Both her B.S. and M.S degree were in Engineering at the KU Leuven (Belgium), after which she obtained a research master at the von Karman Institute. Apart from her main research focus she has a major interest in biodiversity, in particular that of amphibians.



Shashank Balakrishna is a Master's student studying Herpetology at the Vrije Universiteit Brussel, Belgium. He is due to complete his degree in June 2017 with a thesis on the effectiveness of tail autotomy across different landscapes. He is an active member of the Centre for ecological sciences at the Indian Institute of Science, where he works on the local adaptations of lizards from an eco-physiology and behavioral ecology approach within landscape levels. He also interns at the Universiteit Antwerpen where he investigates attributes influencing personality traits in a native and invasive population of *Podarcis muralis*.



David P. Botha is a South African trained conservationist, most recently graduating (2016) from the Vrije Universiteit Brussel, Belgium, with a Master's degree in biology. With a strong background in ecology and a great passion for herpetology, he aims to use multidisciplinary approaches to solve complex questions within these fields. He continues to equip himself with new skillsets that will aid in this endeavour. His main interests lie with the African herpetofauna, particularly snakes and other squamates.



Philippe J. R. Kok is a Belgian evolutionary biologist and herpetologist. He obtained his Ph.D. in biology at the Leiden University (The Netherlands) in 2013. He is currently FWO postdoctoral researcher in the Amphibian Evolution Lab at the Vrije Universiteit Brussel, Belgium, where he also teaches Field Herpetology to the second year Master's students. His interests are eclectic, the main ones being the evolution, systematics, taxonomy, biogeography, and conservation of amphibians and reptiles in the Neotropics, more specifically from the Guiana Shield. His work now primarily focuses on vertebrate evolution in the Pantepui region.



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A new rupicolous species of gecko of the genus *Hemidactylus* Oken, 1817 from the Satpura Hills, Central India

^{1,3}Zeeshan A. Mirza and ²David Raju

¹G-18, 4th A-Cross Rd, Canara Bank Layout, Rajiv Gandhi Nagar, Kodigehalli, Bengaluru, Karnataka 560097, INDIA ²Singinawa Jungle Lodge, Tehsil Baihar, Balaghat, Kohka 481111, Madhya Pradesh, INDIA

Abstract.—We here describe a new species of rupicolous gecko from the Satpura Hills of central India. The new species is a member of the *Hemidactylus brookii* complex, and can be distinguished based on the following suite of characters: moderate sized species (SVL 54.3–74.2 mm); anterior postmental width equal to first infralabial; posterior postmental width equal to second infralabial; posterior postmental not in contact with first infralabial; enlarged, keeled, tubercles, fairly regularly arranged in 15–16 longitudinal rows on dorsum; two angular series of seven precloacal femoral pores separated by diastema of eight non-pored scales; non-pored scales equal to size of pored scales; scales bordering anterior edge of pored scales half the size of pored scales; five lamellae on digit I and seven on digit IV of manus as well as pes; lamellae on digit IV and V of pes absent on basal 25% of the digit; legs long and slender; ventral aspect of tail with broad caudal scales covering ~80% of tail; two subconical post cloacal spurs, anterior spur slightly larger than posterior spur.

Key words. *Hemidactylus brookii*, complex, taxonomy, bPTP, multivariate analysis, DNA

Citation: Mirza ZA, Raju D. 2017. A new rupicolous species of gecko of the genus *Hemidactylus* Oken, 1817 from the Satpura Hills, Central India. *Amphibian & Reptile Conservation* 11(1) [General Section]: 51–71 (e137).

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Received: 27 May 2015; **Accepted:** 20 December 2016; **Published:** 24 March 2017

Introduction

The genus *Hemidactylus* Oken, is the second most speciose gekkonid genus in the world, with ~143 species distributed globally (Uetz and Hošek 2016), its diversity being concentrated in the tropics. India is home to at least 29 species (including *H. gleadowi* Murray) and this number is likely to increase with further sampling (Giri 2008; Giri and Bauer 2008; Mirza and Sanap 2014).

During an expedition that led to the discovery of *Eublepharis satpuraensis* (Mirza, Sanap, Raju, Gawai, and Ghadekar 2014), a species of *Hemidactylus* was collected from Pachmarhi town. Superficially resembling members of the *Hemidactylus brookii* complex, it could be separated from most members of the group by the presence of enlarged subcaudal plates on the tail and other morphological characters. *Hemidactylus brookii* Gray has had a complicated taxonomic history and nearly all attempts to resolve the group have thus far failed largely due to the commensal nature of the species, and inadequate sam-

pling effort in terms of specimens and collection localities, (Bauer et al. 2010a; Mahony 2011; Rösler and Glaw 2010) and a long list of synonyms that remained unaddressed until recently. Mahony (2011) made an effort to address the taxonomic status of several synonyms and his compilation serves as a vital resource for members of this group. However, a few issues remain, for example, the obscure identity of *Hemidactylus brookii* sensu stricto, with Grays specimens being the only true exemplars of the species, as pointed out by Kathriner et al. (2014). Lajmi et al. (2016) presented a comprehensive analysis of the group in India using molecular as well as morphological data. However, due to lack of material from Pakistan, the resolution of the entire species complex is incomplete. Despite the controversial taxonomic nature of members of this group, there are several distinct morphotypes within the complex (see Kathriner et al. 2014; Lajmi et al. 2016; Mahony 2009) that need to be addressed to help resolve the systematics of the entire *H. brookii* complex.

Correspondence. ³snakeszeeshan@gmail.com

Following the key provided by Lajmi et al. (2016), the specimens of *Hemidactylus* from Satpura Hills show affinity to members of clade 4/*H. cf. murrayi*. Multivariate analysis and molecular data support the distinctiveness of the specimens of *Hemidactylus* obtained from Satpura, which we herein describe as a new species. A molecular phylogenetic analysis based on 302 bp of cytochrome *b* gene suggests that the new species is sister to *H. treutleri* Mahony.

Material and Methods

Morphological and meristic data: Specimens in the field were captured by hand and euthanized, followed by fixation in 4% formaldehyde buffer. The specimens were later washed to remove traces of formalin, stored in 70% ethanol, and deposited in the collection of the National Centre for Biological Sciences, Bangalore, India and the Bombay Natural History Society, Mumbai, India. All measurements were taken following Giri and Bauer (2008) with Mitutoyo™ digital calipers (to the nearest

0.1 mm): snout-vent length (SVL: from tip of snout to vent), trunk length (TRL: distance from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hind limb insertion), body width (BW: maximum width of body), crus length (CL: from base of heel to knee); tail length (TL: from vent to tip of tail), tail width (TW: measured at widest point of tail); head length (HL: distance between retroarticular process of jaw and snout-tip), head width (HW: maximum width of head), head height (HH: maximum height of head, from occiput to underside of jaws), forearm length (FL: from base of palm to elbow); ear length (EL: longest dimension of ear); orbital diameter (OD: greatest diameter of orbit), nares to eye distance (NE: distance between anteriormost point of eye and nostril), snout to eye distance (SE: distance between anteriormost point of eye and tip of snout), eye to ear distance (EE: distance from anterior edge of ear opening to posterior corner of eye), internarial distance (IN: distance between nares), interorbital distance (IO: shortest distance between left and right supraciliary scale rows) [Table 1]. Morphological and mor-

Table 1. Morphometric and mensural data for *Hemidactylus chipkali* sp. nov.

Specimen number	Holotype NCBS AT107	Paratype NCBS AT108	Paratype NCBS AT109	Paratype BNHS 2427	Paratype BNHS 2426
Sex	♂	♀	♂	♀	♂
SVL	74.2	65.6	60.1	61.7	54.3
TRL	26.7	26	24.1	25.8	23.7
BW	11.7	13.9	12.0	12.5	10.4
CL	10.8	11.2	10.5	11.7	10.1
TL	59.4	60.4	37.6*	70.7	50*
TW	5.4	5.9	7.7	6.6	6
HL	13.4	16.7	18.4	17.5	17
HW	12.5	12.2	12.4	11.5	10.9
HH	6.5	5.9	7.1	5.5	5.4
FL	8.9	9.2	10.3	9.4	8.5
OD	4.0	3.5	3.2	3.3	3.6
NE	5.5	5.8	6.3	5.0	5.3
SE	7.2	7.9	7.	6.4	6.8
EE	4.4	5.0	4.9	4.8	4.3
EL	1.2	1.3	1.3	1.2	1.2
IN	1.4	1.5	1.5	1.5	1.2
IO	4.1	4.5	4.3	4.0	4.1
Lamellae L manus	5-6-7-7-6	5-7-7-7-8	5-7-7-7-7	5-7-7-7-7	5-7-7-7-7
Lamellae R manus	5-7-7-7-7	5-7-7-7-8	5-7-7-7-7	5-7-7-7-7	5-7-7-7-7
Lamellae L pes	5-7-7-7-7	5-8-8-8-7	5-7-8-7-6	5-7-8-7-7	5-7-8-7-7
Lamellae R pes	5-7-8-7-6	5-8-8-7-7	5-7-8-7-7	5-7-8-7-7	5-8-8-7-7
Supralabials Left	12	10	11	10	10
Supralabials R	11	11	11	11	10
Infralabials L	9	9	9	10	8
Infralabials R	10	9	10	9	8
Pores L/R	7/7	-	8/8	-	8/8
gap between pores	8	-	8	-	8

phometric data for *Hemidactylus brookii* group was obtained from, Lajmi et al. (2016), Mahony (2011), Rösler and Glaw (2010). Principal Component Analysis (PCA) was performed to further support the distinctiveness of the new species. Meristic counts and external observations of morphology were made using a LeicaTM S8A-PO dissecting microscope. Images of the specimens were taken with a CanonTM 70D mounted with a CanonTM 100 mm macro illuminated with two external CanonTM 430EX-II flashes, and plates were edited in Adobe® Photoshop CS5 (<http://www.adobe.com/legal/permissions/trademarks.html>). Institutional acronyms used in the manuscript are as follows: BNHS (Bombay Natural History Society), Mumbai; NHM (Natural History Museum London); NCBS (Collection facility, National Centre for Biological Sciences), Bangalore; ZSI (Zoological Survey of India), Kolkata.

Molecular methods and analysis: Genomic DNA for a single specimen was extracted from tail tissue using the Phenol-Chloroform-Isoamyl Alcohol method, following Sambrook et al. (1989). Partial mitochondrial cytochrome b gene was amplified using primer CytbF700 (5'-CTTCCAACACCAACATCTCAGCATGATGAAA-3') and CytbR700 (5'-ACTGTAGCCCCCTCAGAATGATATTTGTCCTCA-3') published by Bauer et al. (2007). Polymerase Chain Reaction protocols were as followed by Mirza and Patel (2017). The PCR product was cleaned, and sequenced with a 3730 DNA Analyzer after cleaning. The sequence was cleaned manually in MEGA7 (Kumar et al. 2016). In order to ascertain phylogenetic position of the new species, published sequences were retrieved from GenBank used by Lajmi et al. (2016) listed in Appendix I. Sequences were aligned in Mega7 using ClustalW (Thompson and Gibson 2002) with default settings. For optimal partitioning strategy and evolutionary substitution model, aligned data was analyzed using PartitionFinder v.1.1.1 (Lanfear et al. 2012). Maximum Likelihood method was implemented to assess phylogenetic relationship with RAxML (Silvestro and Michalak 2012). Data were partitioned into three codons and GTR+G was used as the sequence substitution model, based on the optimal partitioning scheme suggested by PartitionFinder. Maximum likelihood analysis was run for 1,000 non-parametric bootstrap replicates with rapid ML search option. Sequence divergence uncorrected “p-distance” was calculated in Mega7. Sequence for the new species has been deposited with GenBank accession number “KX044190” for the specimen NCBS AT110.

Species delimitation: Bayesian Poisson Tree Process (bPTP) based on evolutionary placement algorithm was implemented using the web server (<http://species.h-its.org/ptp/>) following Zhang et al. (2013) for inferring putative species. Maximum likelihood tree was supplied for the analysis. Outgroup, *Hemidactylus frenatus*, was ex-

cluded from the analysis for optimum results. The analysis was run for 100,000 generations with three chains and 25% of the trees were discarded as burn-in. Results of the analysis are presented in Appendix III and Appendix IV.

Systematics

Hemidactylus chipkali sp. nov.

Fig. 1–5, Table 1.

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Holotype: NCBS AT107, adult male, from a cliff along the road leading to Pachmarhi town, Hoshangabad District, Madhya Pradesh (22.485050°, 78.449340°, 1,092 m). Collected on 09 May 2014 by Rajesh Sanap, David Raju, and Zeeshan Mirza.

Paratypes (four specimens): NCBS AT109 and BNHS 2426, adult males; NCBS AT108 and BNHS 2427, adult females, same data as holotype.

Diagnosis: A moderate sized species of the genus measuring 54.3–74.2 mm; TRL/SVL 36–43.6%; HL/SVL 26–31%; ear opening oval; anterior postmental width equal to first infralabial; posterior postmental width equal to second infralabial, posterior postmental not in contact with first infralabial; enlarged, keeled, tubercles, fairly regularly arranged in 15–16 longitudinal rows on dorsum; two angular series of seven precloacal femoral pores on each side separated by diastema of eight non-pored scales; non-pored scales equal to size of pored scales; scales bordering anterior edge of pored scales half the size of pored scales; five lamellae on digit I and seven (rarely eight) on digit IV of manus as well as pes; lamellae on digit IV and V of pes absent on basal ~25% of the digit; limbs long and slender FL/SVL 0.15 and CL/SVL 0.18; ventral aspect of tail with broad caudal scales covering ~80% of the tail; two subconical post cloacal spurs, anterior spur slightly larger than the posterior spur.

Etymology: The specific epithet “*chipkali*” is the Hindi word for gecko.

Description of holotype male NCBS AT107: Holotype in good condition preserved in a linear manner with a slightly curved tail. Hemipenis partly everted. Over half of the tail regenerated (Fig. 1A, B).

A medium sized gecko (SVL 59 mm) with a fairly large head (HL/SVL ratio 0.23), head slightly longer than wide (HW/HL ratio 0.92), head slightly depressed (HH/HL ratio 0.48), distinct from neck (Fig. 2A); canthus rostralis slightly inflated; snout short (SE/HW ratio 0.57), obtusely pointed from dorsal view and acutely in lateral view (Fig. 2B), longer than eye diameter (OD/SE ratio 0.55); scales on the snout subequal, convex, those anterior to the eye and on canthus rostralis, larger than the surrounding scales; eyes large (OD/HL ratio 0.29), pupil vertical with crenulated edges; supraciliaries smaller on



Fig. 1. *Hemidactylus chipkali* sp. nov. male holotype NCBS AT107, (A) dorsal view, (b) ventral view.

the anterior edge of the orbit, gradually increasing in size as they progress towards upper surface of the head; ear-opening large, sub-oval, obliquely oriented, its length at its greatest extent thrice that of the orbital diameter (EL/OD ratio 0.30) bearing three lobules on its anterior inner wall; eye to ear distance greater than diameter of eye (EE/OD ratio 1.11); rostral quadrangle, much wider than deep, divided by a median suture for its entire length; rostral in contact with nasal, first supralabial and internasals; two large and a slightly smaller internasals between nasals; mental triangular, wider (3.2) than long (2.8); two pairs of postmentals, anterior postmental longer (2.6) than wide (1.8); posterior pair of postmental slightly smaller than anterior pair, longer (2.3) than wide (1.4); anterior postmental in contact with mental, infralabials and posterior pair of postmental; posterior postmentals separated by five scales; anterior postmental equal in width to the first infralabial; posterior postmental equal to width of second infralabials (Fig. 2C); scales on throat circular, smaller than the ones ventral aspect of trunk; supralabials (to midorbital position) nine on left and ten on right side; supralabials (to angle of jaw) eleven on left side and twelve on right side; infralabials (to angle of

jaw) nine on left and ten on right side.

Body elongate (TRL/SVL ratio 0.45) and dorsoventrally flattened, more so after preservation; lacking distinct ventrolateral furrow; dorsal scalation on trunk granular intermixed with enlarged, keeled, tubercles, fairly regularly arranged in 15–16 longitudinal rows; dorsal tubercles on mid-dorsum longer (1.0) than wide (0.9); individual tubercle row separated from the adjacent by three transverse scale rows and by preceding tubercle of the same row, by four scale rows (Fig. 3A); ventral scales on trunk smooth, flat, larger than dorsal scales; mid body scales across belly 28–30 (Fig. 1B); eight (left) and seven (right) femoral pores separated at mid-pelvic region by eight non-pored scales; non-pored scales slightly larger than pored scales (Fig. 3B).

Limbs moderately long, slender; digits dilated, bearing slightly oblique lamellae on ventral surface; clawed, claw nearly half the length of the lamellar region; forelimbs short (FL/SVL ratio 0.15), slightly shorter than hind limbs (CL/SVL ratio 0.18), all digits of manus and digits I–IV of pes indistinctly webbed at the base. Terminal phalanx of all digits curved, arising angularly from distal portion of expanded lamellar pad, free portion of

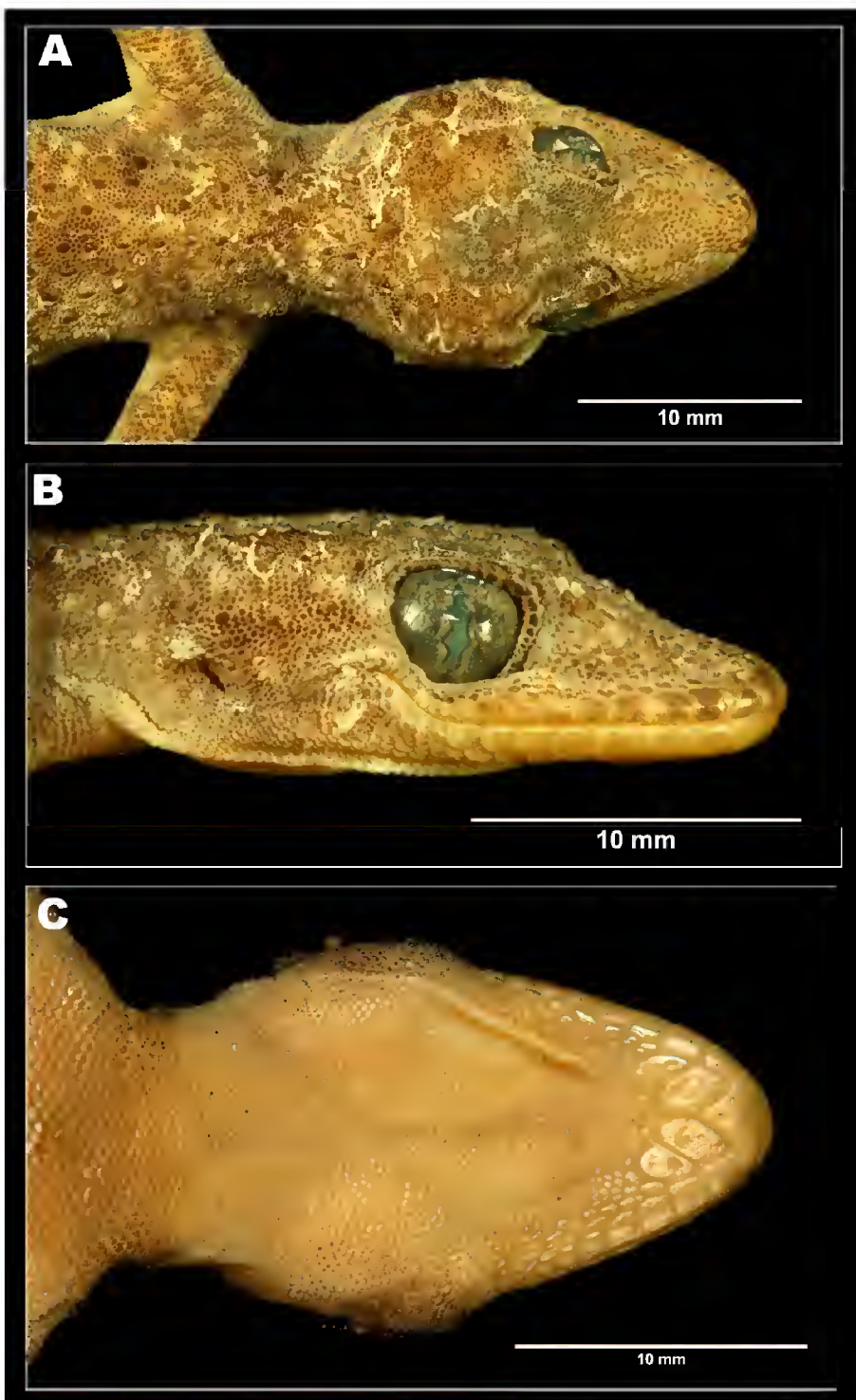


Fig. 2. *Hemidactylus chipkali* sp. nov. male holotype N.CBS AT107 head, (A) dorsal view, (B) lateral view, (C) ventral view.

phalanx of all digits half to more than half long as the dilated portion. Lamellae beneath the digits, left manus 5-6-7-7-6, right manus and left pes 5-7-7-7-7 (Fig. 4A), right pes 5-7-8-7-6 (Fig. 4B). Lamellae not reaching the base of the digit IV of pes. Relative lengths of digits: III>IV=V>II>I (right manus), IV>II=III>V>I (right pes). Tail moderately depressed, oval in cross section, longer than snout-vent length (TL/SVL ratio 1.26), 59.4 mm of the tail regenerated. Caudal segments distinct; pholidosis of original tail dorsum with small, juxtaposed scales intermixed with large depressed keeled tubercles, scales on regenerated portion of tail with slightly larger scales and lacking tubercles. First tail segment with a whorl of ten large conical, keeled tubercles, second segment onwards, each segment with six tubercles. Ventral aspect with large, broad scales covering about ~80% of the tail width from base of tail to the tip (Fig. 3C). Two subconical post cloacal spurs, anterior spur slightly larger than the posterior spur.

Coloration in life (Fig. 5): Coloration is a shade of pale brown with white and dark brown spots across the dorsum. Three adjoining brown spots over the nape and similar spots at a fairly regular distance from the preced-

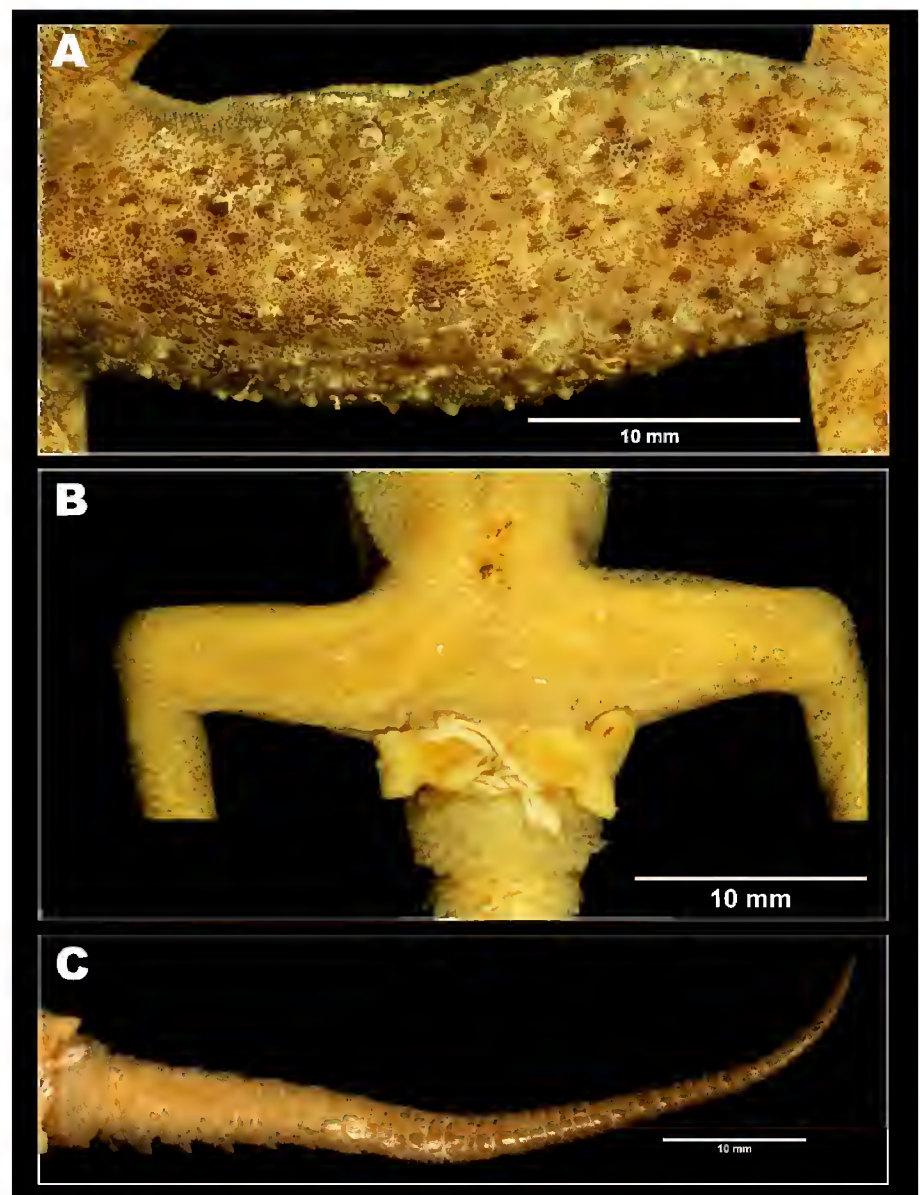


Fig. 3. *Hemidactylus chipkali* sp. nov. male holotype NCBS AT107, (A) dorsal view of trunk, (B) ventral view of cloacal showing precloacal femoral pores and hemipenis, (C) ventral view of tail showing enlarged sub-caudal scales.

ing row all through the trunk up to the flank. Each dark band is separated from the subsequent one by loosely connected white spots, to form thin white bands. Limbs pale brown with whitish spots all over the dorsum. Tail with alternate pale brown and cream colored bands. Coloration in preservative (Fig. 6): Specimens paler than in life with vestigial remains of dark brown spots and little to no trace of white spots on dorsum. Underparts straw colored.

Natural History: The new species was collected from vertical cliffs along a road leading to Pachmarhi situated in the Satpura Hills of central India (Fig. 7). Satpura Hills are located south of the Narmada River, running parallel to the river from western Gujarat through the borders of Maharashtra and Madhya Pradesh, extending up to northeastern Madhya Pradesh. The landscape at the type locality is undulating with the highest peak reaching 1,350 m and is dominated by tree species like *Tectona grandis* and *Shorea robusta*, characteristic of deciduous forests in the region. The hills at the type locality have steep cliffs where the new species was found (Fig. 8). All the type specimens and a few additional uncollected specimens were found actively moving on roadside rocks (Fig. 9). The species was observed to be sympatric with *Eublepharis satpuraensis*, which likely shares the crevices in the cliff during the day and emerges at dusk. Most individuals would dart towards the nearest crevice when

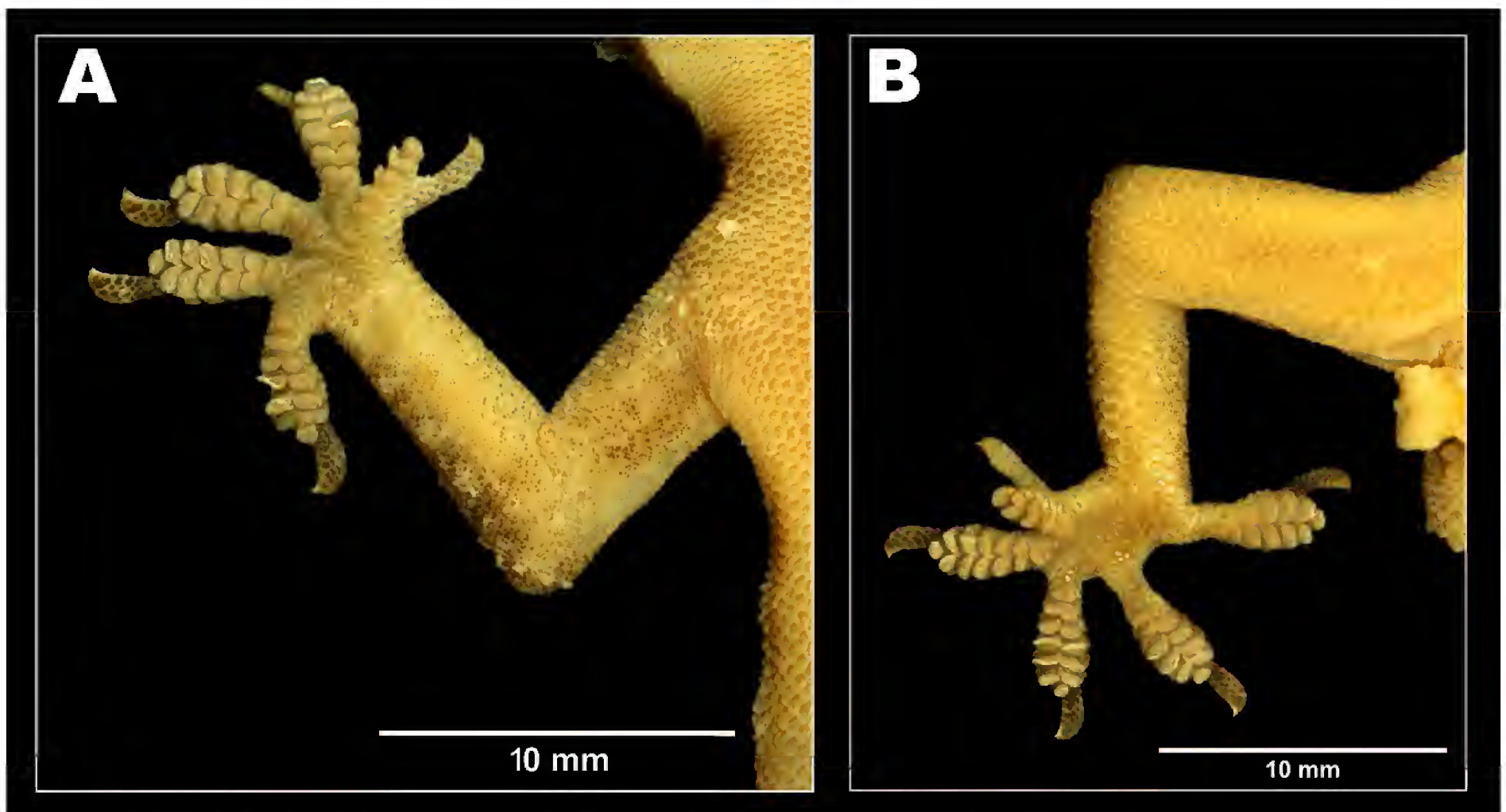


Fig. 4. *Hemidactylus chipkali* sp. nov. male holotype NCBS AT107 (A) right manus, (B) right pes.

approached with flashlights. The new species is likely restricted to these high elevation cliffs as only *H. cf. gleadowi* was recorded from near Bijakhori village, Sohagpur ca. 30 km (aerial distance, elevation 367 m) northwest of the type locality. One of the female paratypes (NCBS AT108) shows presence of two well-developed eggs within the body cavity suggesting that the species breed during the summer. With regards to its IUCN status, we recommend designating this species as “Data Deficient” in terms of its distribution, until further reports surface. During the course of the survey, no immediate threat to the species was observed and the entire area where the species was observed is protected as part of the Satpura Tiger Reserve. However, large parts of the Satpura hills are not protected and further surveys must be conducted to assess conservation threats to the species outside protected areas.

Suggested common name: Central Indian Leaf-toed Gecko.

Comparison: *Hemidactylus chipkali* sp. nov. differs from most Indian congeners in bearing the following set of differing and non-overlapping characters: SVL 54.3–74.2 mm (vs. SVL > 80 mm in *H. maculatus* Duméril and Bibron, *H. graniticolus* Agarwal, Giri, and Bauer, *H. giganteus* Stoliczka, *H. gujaratensis* Giri, Bauer, Vyas, and Patil, *H. prashadi* Smith, *H. acanthopholis* Mirza and Sanap, *H. aaronbaueri* Giri, *H. yajurvedi* Murthy, Bauer, Lajmi, Agarwal, and Giri), dorsum with keeled tubercles fairly arranged in 15–16 longitudinal rows (vs. tubercles absent or few or irregularly arranged in *H. aequilonius* Zug and McMahan, *H. flaviviridis* Rüppell, *H. frenatus* Schlegel, *H. garnotii* Duméril and Bibron, *H.*

leschenaultii Duméril and Bibron, *H. hemchandrai* Dandge and Tiple), presence of seven femoral pores separated medially by a diastema of eight non-pored scales (vs. precloacal pores in *H. sataraensis* Giri and Bauer, *H. gracilis* Blanford, *H. reticulatus* Beddome, *H. albofasciatus* Grandison and Soman, *H. scabriceps* Annandale, *H. persicus* Anderson, *H. robustus* Heyden, *H. turcicus* Linnaeus), dorsal tubercles sub-triangular (vs. tubercles triangular in *H. triedrus* Daudin and *H. subtriedrus* Jerdon), lacking webbing on hind feet and a fringe of skin on lateral aspect of tail (vs. present in *H. platyurus* Schneider), lamellae divided (undivided in *H. anamallensis* Günther).

Hemidactylus chipkali sp. nov. is most similar to members of the *Hemidactylus brookii* group and is here compared with taxa considered valid by Mahony (2011) and Kathriner et al. (2014). *Hemidactylus chipkali* sp. nov. differs from *H. brookii* (as defined by Mahony 2011) in larger SVL 74 mm (vs. SVL 55.8 mm in *H. brookii*, SVL 43.1 mm in *H. gleadowi* Murray, SVL 45–51.4 mm in *H. kushmorensis* Murray, 51.5 mm in *H. parvimaculatus* Deraniyagala); anterior postmental width equal to first infralabial; posterior postmental width equal to second infralabial, posterior postmental not in contact with first infralabial or with narrow contact Figs. 10A–E (vs. width of posterior postmental shorter than width of second infralabial in *H. brookii*, width of posterior postmental shorter than width of second infralabial, posterior in contact with first infralabial in *H. gleadowi*, anterior, posterior postmental in broad contact with first infralabial in *H. kushmorensis*, width of anterior postmental longer than first infralabial, and anterior postmental in contact with first and second infralabials in *H. treutleri* Mahony); seven precloacal femoral pores separated medially by a

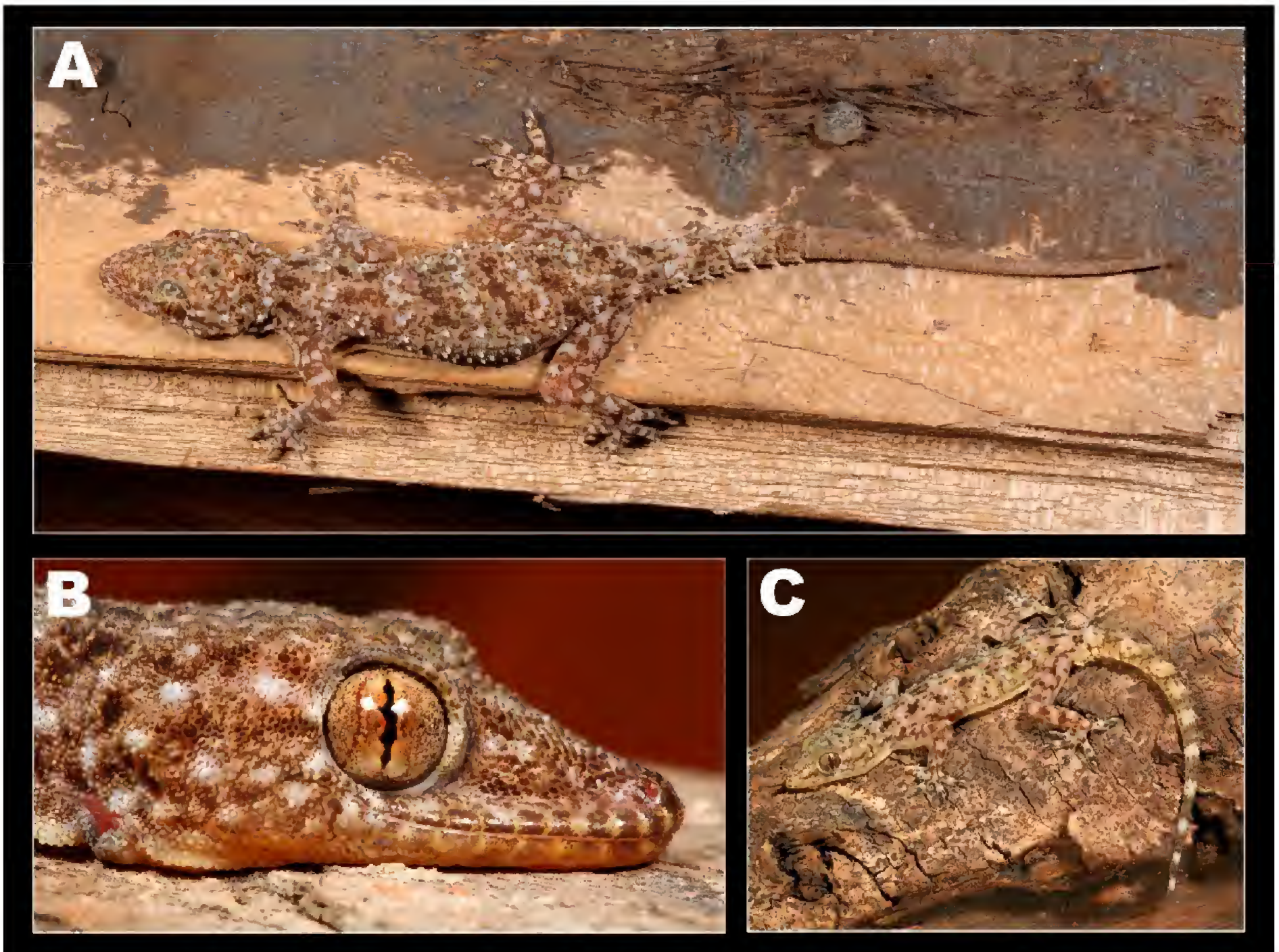


Fig. 5. *Hemidactylus chipkali* sp. nov. (A and B) male holotype NCBS AT107 in life, (C) male paratype NCBS AT108 in life.



Fig. 6. Dorsal aspect of the type series showing coloration after preservation.

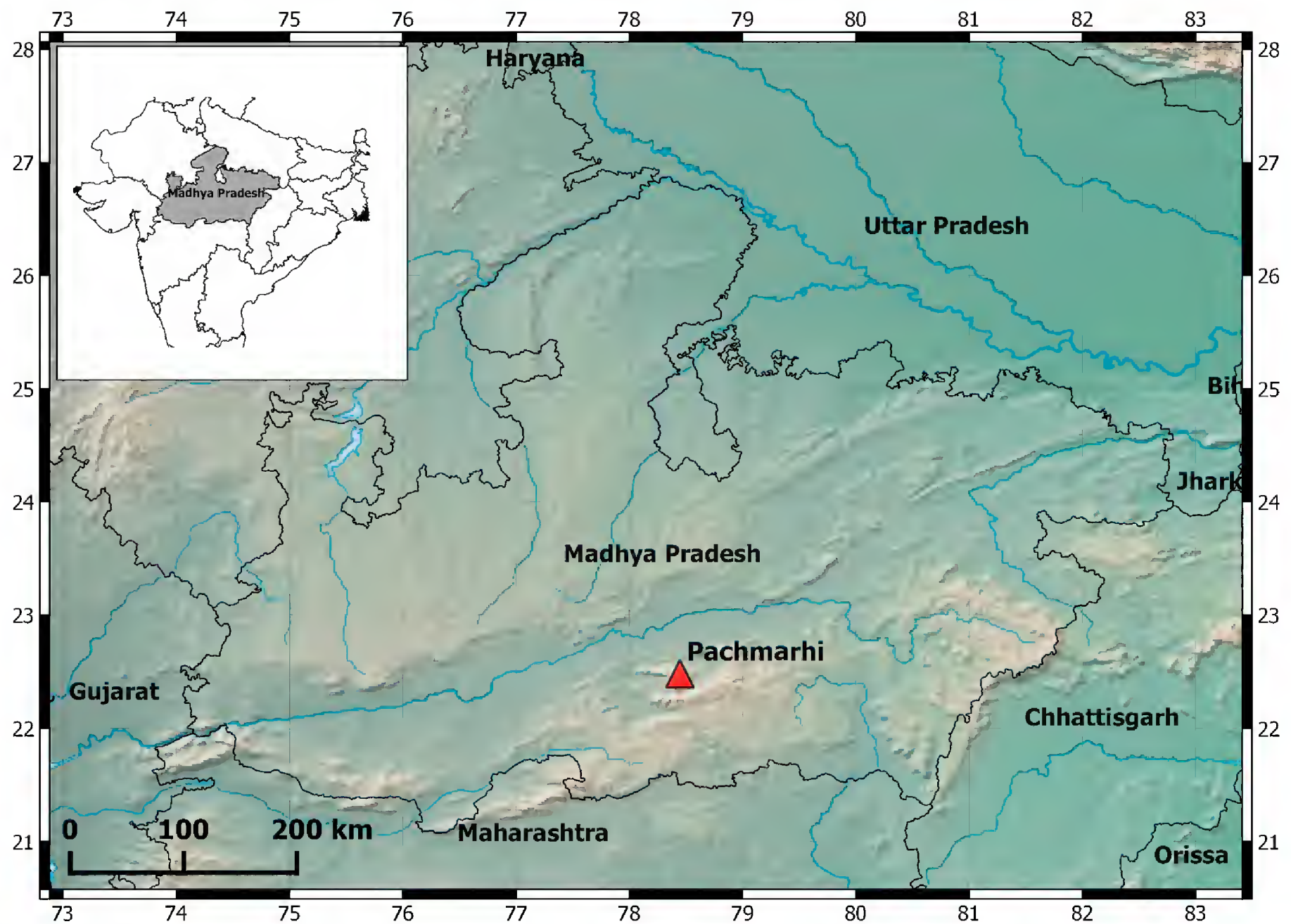


Fig. 7. Map of Madhya Pradesh, central India, showing the type locality Pachmarhi (indicated by a red triangle). Inset map shows location of Madhya Pradesh in India.



Fig. 8. Biotope of Satpura hills showing characteristic rocky cliffs and forest cover where the new species was collected.



Fig. 9. Rocks along the road leading to Pachmarhi town where the type specimens were collected.

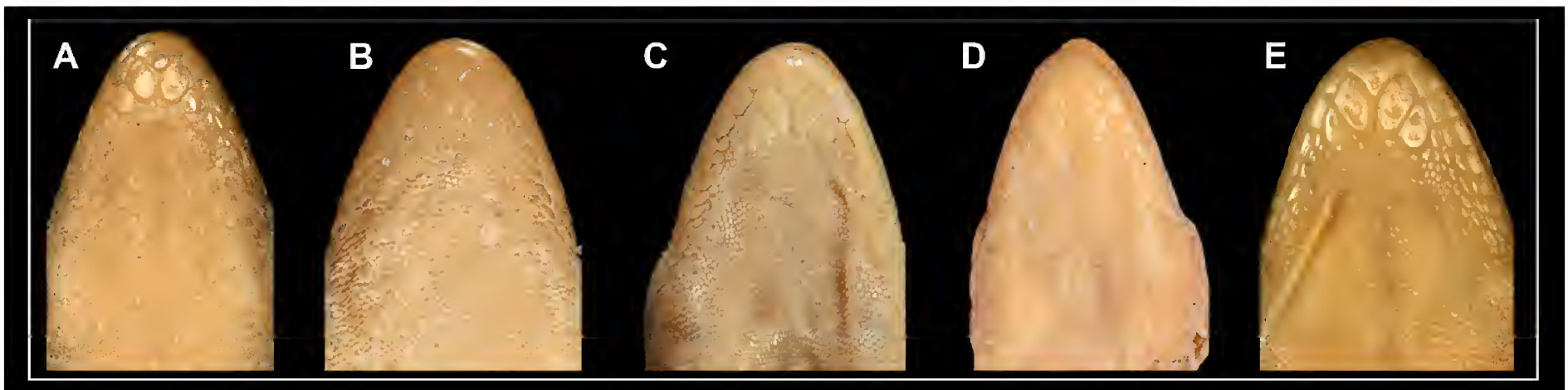


Fig. 10. Ventral view of head, (A) *H. cf. gleadowi* NCBS HA-107, (B) *H. cf. murrayi* NCBS HA-110, (C) *H. subtriedroides* lectotype NHM 1946.8.2554, (D) *H. treutleri* holotype ZSI 25711, (E) *H. chipkali* sp. nov.

diastem of eight non-pored scales Figs. 11A–E (vs. 12–13 precloacal femoral pores separated medially by a diastem of one non-pored scale in *H. brookii* and *H. gleadowi*, 10–11 precloacal femoral pores separated by 2–3 non-pored scales in *H. kushmorensis*, 12–15 pores separated by 2–4 non-pored scales in *H. parvimaculatus*, 7–8 separated by 5–6 non-pored scales in *H. subtriedroides* (Anandale); lamellae series on digit IV do not extend to base Figs. 12A–C (vs. lamellae series cover the entire digit IV in *H. brookii*, *H. cf. murrayi* Gleadow, *H. subtriedroides*, *H. tenkatei* Lidth de Jeude, *H. treutleri* and *H. kushmorensis*); scales bordering anteriorly the precloacal pores half the size of pored scales (vs. scales bordering anteriorly the precloacal pores \geq the size of pored scales in *H. brookii*); keeled dorsal tubercles in 15–16 fairly longitudinal rows (vs. 19–20 in *H. kushmorensis*); five lamellae on digit I of pes (vs. 6–7 in *H. treutleri*); lamellae on digit IV of pes 7 rarely 8 (vs. 10 in *H. kushmorensis*, 8

in *H. cf. murrayi*, 9 in *H. treutleri*, 11 in *H. mahendrai* Shukla); caudal plates enlarged and cover $\sim 80\%$ of the underside of the tail (vs. tail plates not covering entire tail in *H. gleadowi*, *H. kushmorensis*, *H. subtriedroides*, *H. tenkatei*); two sub-conical postcloacal spurs, anterior one slight larger than the posterior (vs. 2–3 medium sized with or without an additional large spur in *H. subtriedroides* and *H. cf. murrayi*, two very small ones in *H. gleadowi*, a single domed spur in *H. kushmorensis*, three enlarged spurs in *H. treutleri*); sub-caudal completely transverse the tail width (sub-caudal not as enlarged as in *H. gleadowi*, *H. kushmorensis*, *H. subtriedroides*, *H. tenkatei*, *H. brookii*, *H. cf. murrayi*), FL/SVL 0.15 (vs. 0.13 in *H. kushmorensis*, *H. subtriedroides*, *H. brookii*, 0.12 in *H. gleadowi*, *H. cf. murrayi*); CL/SVL 0.18 (vs. 0.15 in *H. brooki*, *H. gleadowi*, 0.14 in *H. kushmorensis*, 0.16 in *H. tenkatei*, *H. subtriedroides*).

Discussion and Conclusion

Phylogenetic relationships within Indian and South Asian *Hemidactylus* have been the subject of recent studies (Bansal and Karanth 2010; Bauer et al. 2010b). However, due to lack of extensive sampling in most studies, the *H. brookii* complex remained unresolved and still does. Kathriner et al. (2014) provided new insights on

the systematics of the *H. brookii* complex, which raises doubts on previous assumptions and results based solely on morphology. Lajmi et al. (2016) presented the first ever comprehensive analysis of *H. brookii* complex incorporating molecular as well as morphological data which has enhanced our understanding of this group thus enabling us to present preliminary data on phylogenetic relationship of the new species. Based on a short fragment of ~302 bp of mitochondrial *cyt b* gene, the new species appears to be allied to *H. treutleri* (Fig. 13) and is a member of clade 5 of Lajmi et al. (2016). It however differs in having an uncorrected p-distance of 14–16% (Fig. 14, Table 4). The phylogenetic analysis results presented here are preliminary, based on data generated by Lajmi et al. (2016). Publication by Lajmi et al. (2016) contains 30 accession numbers, which are repeats and is likely a typographical error. The authors have not copied accession number correctly from previous studies and have the same accession numbers for several sequences of specimens from different localities. It is hoped that the authors provide correct accession numbers and their respective voucher details in a subsequent paper.

Relationships recovered from molecular phylogenetics shows discordance with morphology in the new species, showing close affinity to *H. cf. murrayi* based on morphology whereas it shows affinity to *H. treutleri* based on molecular data. The new species however differs from *H. treutleri* and *H. cf. murrayi* in having broad sub-caudals transverse the entire width of the tail and in bearing 7 lamellae on fourth toe vs. 8 in *H. cf. murrayi* and 9 in *H. treutleri*. Principal Component Analysis of data including morphometric data for the new species, *H. treutleri* and *H. cf. murrayi* for standardized morphometric data showed PC1 + PC2 explaining 80% + 16% of variance, respectively (Fig. 14, Appendix II). Plot of the first two principal axes resulted in two clusters; one of *H. chipkali* and another one of *H. treutleri* and *H. cf. murrayi* (Fig. 15). Results from bPTP support the distinctiveness of the species with high support (Appendix III and IV).

Hemidactylus murrayi Gleadow, 1887 was described based on a series of 24 specimens from “Pimpri and Garvi, in the Dangs” in southern Gujarat. The types are like-

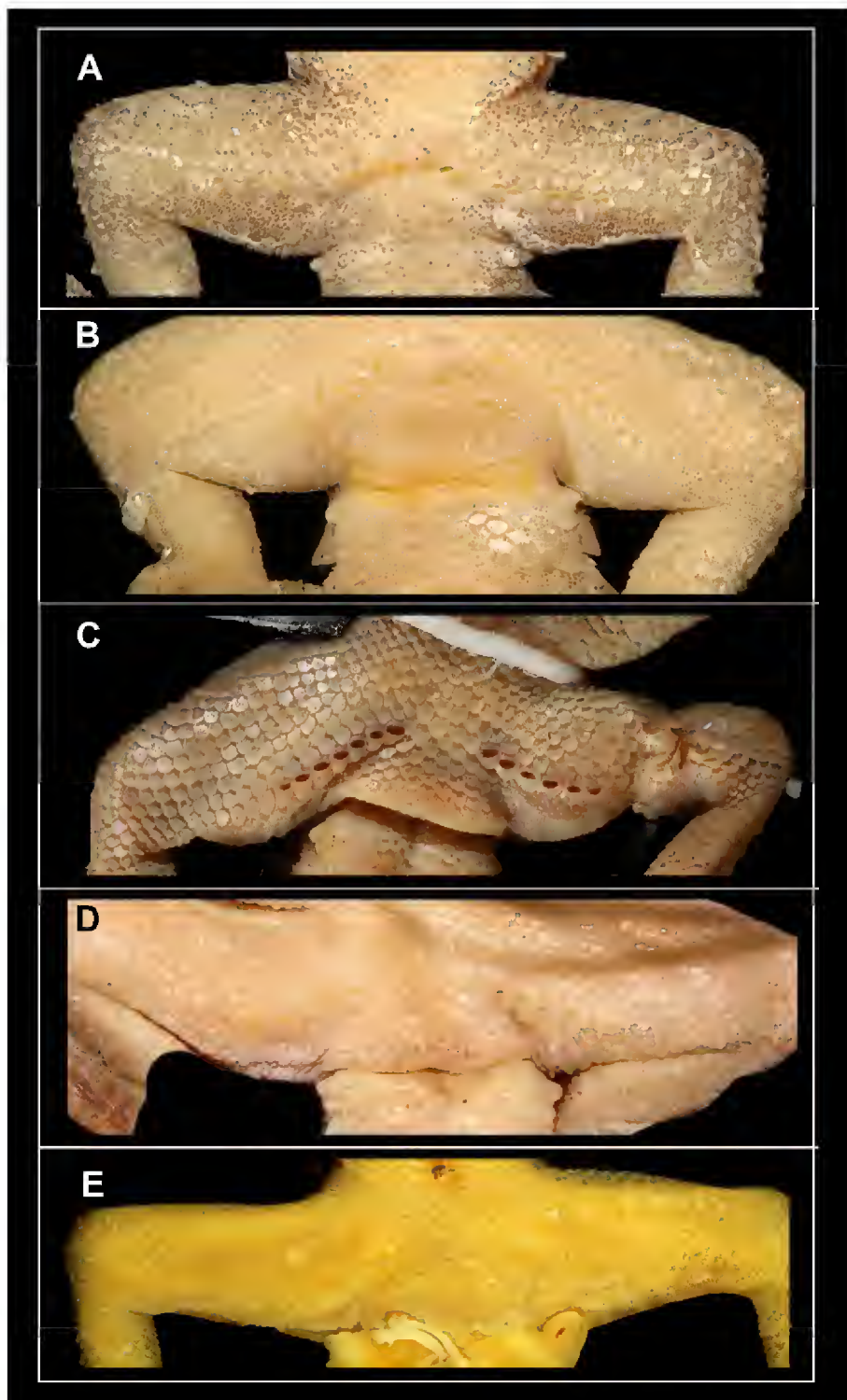


Fig. 11. Precloacal and or femoral pores, (A) *H. cf. gleadowi* NCBS HA-107, (B) *H. cf. murrayi* NCBS HA-110, (C) *H. subtrianguloides* lectotype NHM 1946.8.2554, (D) *H. treutleri* holotype ZSI 25711, (E) *H. chipkali* sp. nov.



Fig. 12. Ventral aspect of right pes, (A) *H. cf. gleadowi* NCBS HA-107 note lamellae on digit IV not reaching base, (B) *H. cf. murrayi* NCBS HA-110 lamellae covering entire digit IV, (C) *H. chipkali* sp. nov. Note lamellae on digit IV not reaching base.

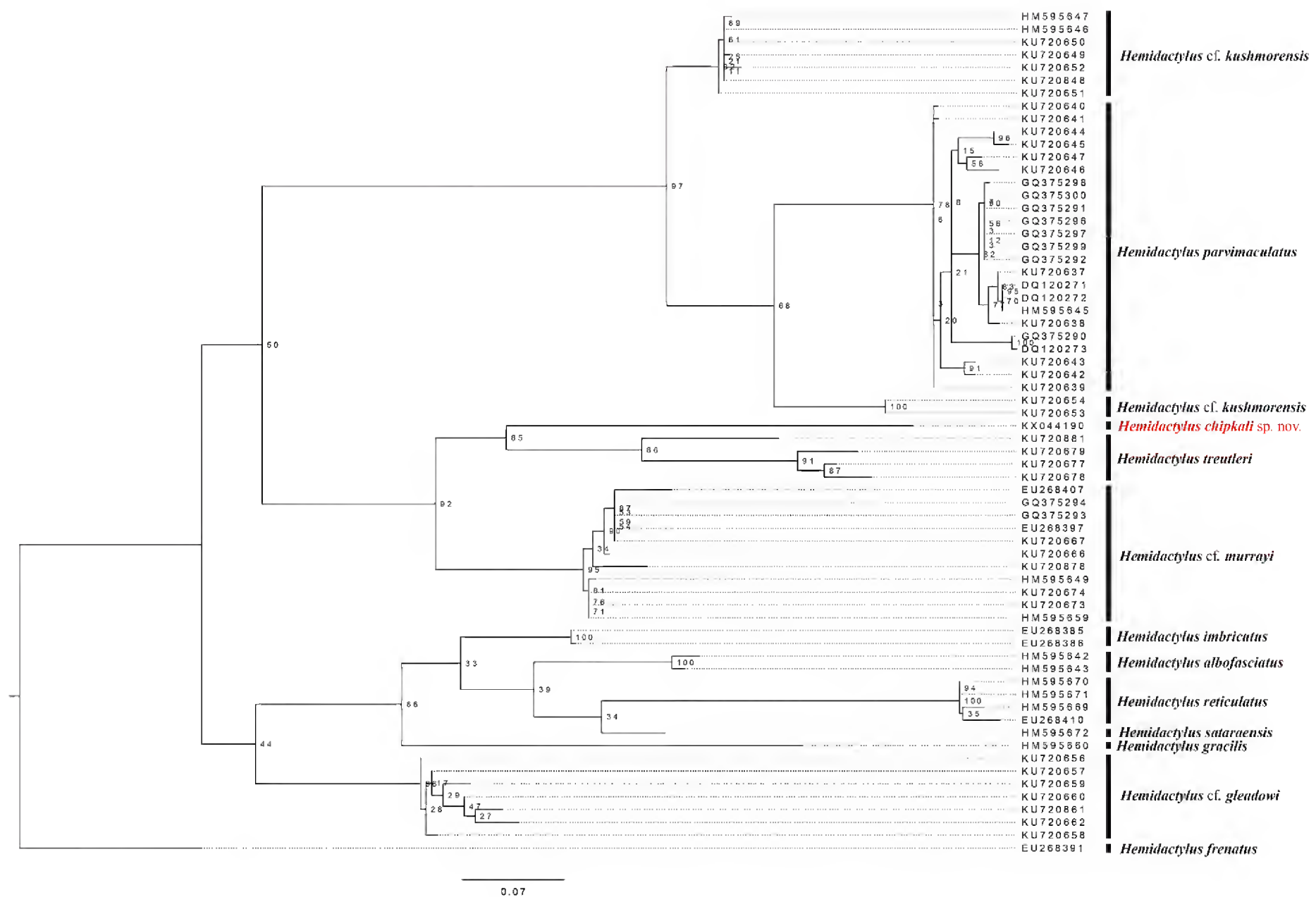


Fig. 13. Maximum likelihood tree for selected members of the *H. brookii* group showing relationship of *H. chipkali* sp. nov. rooted with *H. frenatus* as outgroup based on ~302 bp of mitochondrial cytochrome *b* gene with 1,000 non-parametric bootstrap replicates. Numbers at nodes indicate bootstrap support.

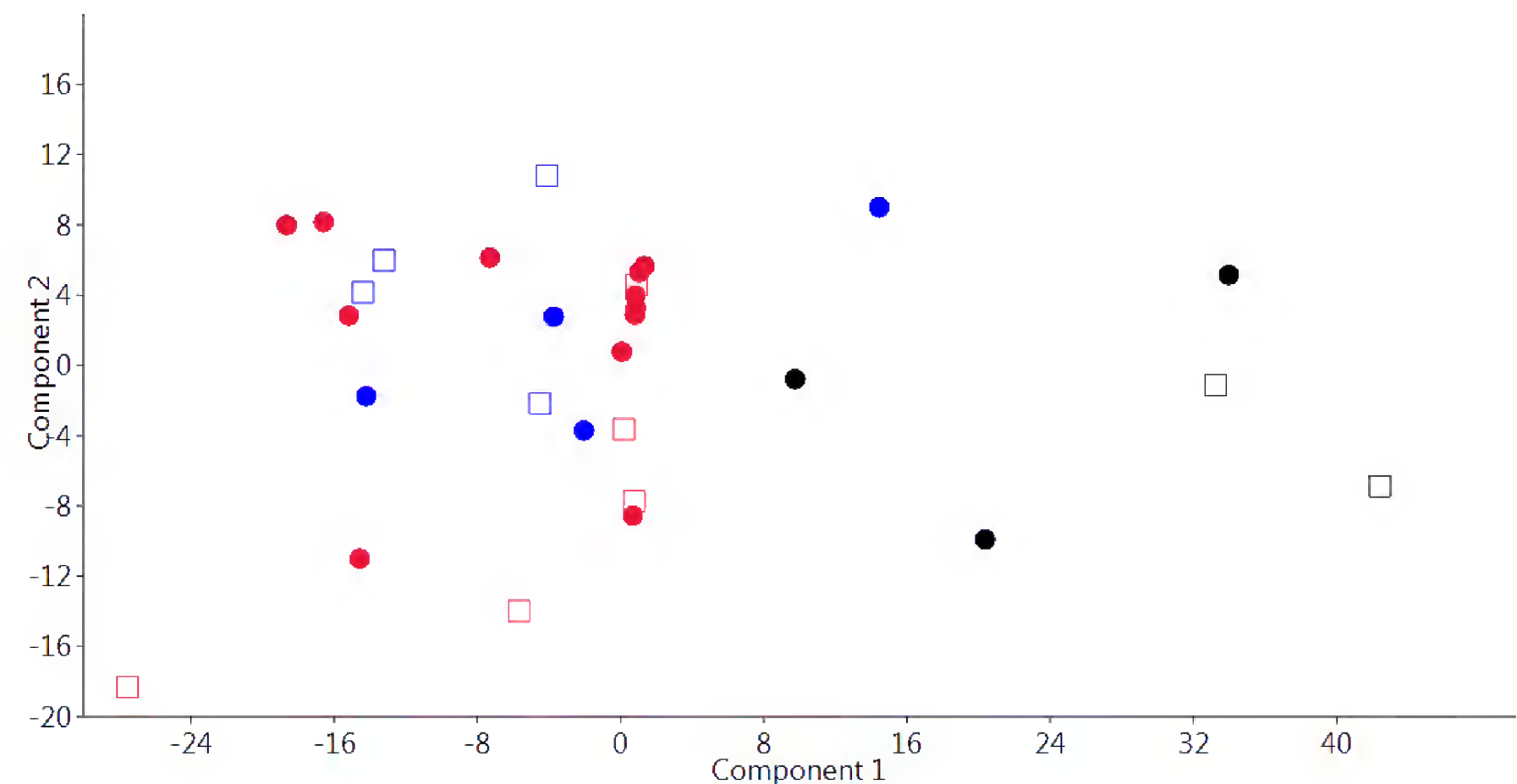


Fig. 14. PCA plot for standardized morphometric data for *H. chipkali* sp. nov. (black), *H. cf. murrayi* (red) and *H. treutleri* (blue). Circles = male and squares = female.

ly lost and hence the identity of the species can only be ascertained after examination of material from both the said localities. Lajmi et al. (2016), who considered clade 4 of their work as *H. murrayi*, however do not include molecular data from either of the localities from where types of *H. murrayi* were collected. *Hemidactylus tenkatei* was found nested in clade 4 of Lajmi et al. (2016), suggesting that this clade might represent more than one species. To further support this, a sample from Nashik (GenBank accession number KU720676) in clade 4 of Lajmi et al. (2016) shows ~8% sequence divergence for cytochrome *b* from other members of the clade which may represent yet another undescribed taxon (Appendix V). Results from bPTP suggests that *H. treutleri* likely is a species complex (Appendix III and IV). In order to resolve this group, a neotype for *H. murrayi* and *H. mahendrai*, each, must be designated and molecular data for the neotypes/topotypes must be incorporated in a phylogeny, and also molecular data from the type locality of *H. subtriadroides* Annandale (suggested to be a valid species by Kathriner et al. 2014), which will shed light on the systematics of this clade that we refer to as *H. cf. murrayi* in the present work. Considering that members of the *H. cf. murrayi* clade are commensal, it may not be an easy task to resolve this complex, largely due to multiple back and forth colonizations in recent years through human agencies.

Description of yet another member of the *H. brookii* complex highlights the diversity of the *H. brookii* complex in India. Including the present description, at least seven species of the *H. brookii* complex have been recorded from India including *H. mahendrai* (see Lajmi et al. 2016). India and Sri Lanka are home to several endemic species of *Hemidactylus* (Bauer et al. 2010b). This number is steadily increasing with dedicated surveys, as well as re-examination of museum specimens (Agarwal et al. 2011; Mirza and Sanap 2014). Explorations of isolated and/or unexplored hill ranges like the Satpura hills, Aravalli hills, and other small hills across the country would certainly harbor undocumented species of reptiles as demonstrated by the discoveries of *Cyrtodactylus srilekhae*, *C. rishivalleyensis*, *C. varadgirii* (Agarwal 2016, Agarwal et al. 2016), *Eublepharis satpuraensis* (Mirza et al. 2014), *H. chipkali* sp. nov., *H. sataraensis* (Giri and Bauer 2008), and *Wallaceophis gujaratensis* (Mirza et al. 2016). Our finding further attests the poor nature of reptilian documentation in the country and the lack of taxonomic revisions on most reptilian groups (see Gowande et al. 2016, Mirza and Sanap 2014, Mirza et al. 2010).

Acknowledgments.—This herpetofaunal documentation project would not have been possible without necessary permits granted by the forest department of Madhya Pradesh for which we are grateful to Narendra Kumar (PCCF, Wildlife and Chief Wildlife Warden), Ravi Shrivastava (PCCF Wildlife), R.P. Singh (APCCF Wildlife),

Anil Nagar (Field Director, Satpura Tiger Reserve), and Dr. Suhas Kumar for necessary permissions to carry out research in Madhya Pradesh. We thank Tulika Kedia and Singinawa Conservation Foundation for all their help and support. We also thank Forsyth Lodge for logistic support and permission to conduct surveys on their property. Rahul Khot (BNHS, Mumbai), Kaushik Deuti (ZSI, Kolkata), and Patrick Campbell (NHM, London) helped with access to type specimens. Special thanks goes to Anurag Mishra (NCBS) for reviewing the final draft of the manuscript. Harshil Patel provided valuable input on *H. cf. murrayi* for which we kindly acknowledge him. Rajesh Sanap and Surya Ramachandran in addition helped with fieldwork. Krushnamegh Kunte granted permission and access to lab facilities at the National Centre for Biological Sciences. Fieldwork was supported by a generous grant from the Rufford foundation to ZM. Special thanks to Robin K. Abraham for constructive comments on the final draft of this paper. Finally, I. Das and Ishan Agarwal provided valuable comments from which the manuscript greatly benefitted.

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Zeeshan A. Mirza is an independent researcher presently pursuing a Master's degree in zoology from Mumbai University. He has largely been interested in the taxonomy of snakes, lizards, and arachnids. Zeeshan has published several papers on their taxonomy including descriptions of over thirty new species. He received a Bachelor's degree in zoology from Bhavans College, Mumbai University. He was born in Mumbai city and has worked largely on the city's reptilian diversity and has worked on several projects to document reptiles and arachnids of Western Ghats. He plans to pursue his further studies on systematics of reptiles and arachnids with an integrated approach involving molecular and morphological data.



David V. Raju comes from a small village called Kuzhimattom in Kottayam District of Kerala, India. He graduated from Baseli College Kottayam in English Literature. David has always had an interest in wildlife and further developed his interest at the Kottayam Nature Society. Currently he is working as a naturalist in Central India. He has co-authored a book on the dragonflies of Kerala. He was also a part of the team which discovered ten frogs in Western Ghats and a leopard gecko from Satpura hills. His other interests are birds, butterflies, and mammals.

Comperative material examined:

Hemidactylus aaronbaueri: Holotype BNHS 1739 (male), Ghatghar, Taluka Junnar, Pune District, Maharashtra, India; ZSI 21648A and ZSI 21648C (female), ZSI 21648B (male), Bhairavgadh Fort, Taluka Karjat, Pune District, Maharashtra, India.

Hemidactylus acanthopholis: Holotype NHM 1946.8.23.68 (male), paratypes NHM 1946.8.23.67 (male), Tirunelveli District, Tamil Nadu.

Hemidactylus albofasciatus: Paratype ZSI 21109 (female), Dorle village, Rajapur Taluka, Ratnagiri District, Maharashtra, India; two males BNHS 1579 and 1582 Dabhil-Ambere, Ratnagiri District, Maharashtra, India.

Hemidactylus bengaliensis Anderson (= *H. flaviviridis*): Syntype ZSI 5780, Bengal.

Hemidactylus flaviviridis: ZSI 20963 (male) Jaipur, Rajasthan, India; ZSI 21688 (female) Udaipur, Rajasthan, India.

Hemidactylus hunae: Type specimen NHM 1946.8.23.77 (female), Okanda, Eastern Province, Sri Lanka.

Hemidactylus giganteus: Syntype, NHM 1877.8.6.5 (male), Godavari valley near Bhadrachalam, Andhra Pradesh, India.

Hemidactylus cf. *gleadowi*: Male NCBS HA-107 Khamgaon, Maharashtra; male NCBS HA-108 Sohagpur, Madhya Pradesh.

Hemidactylus gracilis: Syntype, ZSI 5190 (male), from “S.E. Berár” (in Madhya Pradesh, India; BNHS 1591 (male) and BNHS 1592 (female), Chatushringi hills, Pune, Maharashtra, India.

Hemidactylus graniticolus: Holotype BNHS 1850 (female), hills near Harohalli, Bangalore Rural district, Karnataka, India; paratypes NHM 1946.8.23.70 (female), NHM 1946.8.23.71 (female), NHM 1946.8.23.72 (male), NHM 1946.8.23.73 (female), NHM 1946.8.23.74 (female), NHM 1946.8.23.75 (female), Salem District, Tamil Nadu, India; NHM 1946.8.23.76 (male), “Malabar,” India.

Hemidactylus gujaratensis: Holotype BNHS 1818 (female) Vagheshwari Mata Temple, Junagadh City, Junagadh District, Gujarat, India.

Hemidactylus kelaartii: Syntypes ZSI 2617 (male) and ZSI 2618 (female), from “Ceylon” (= Sri Lanka).

Hemidactylus maculatus: NHM 1956.1.11.41 (female), Matheran, Raigad District, Maharashtra, India; ZSI 25608 (male) Government rest house, Panchagani, Satara District, Maharashtra, India; BNHS 74 (female) and BNHS 75 (female), Mumbai, Maharashtra, India; BNHS 1086 (male), Kanheri caves, Mumbai, Maharashtra, India.

Hemidactylus marmoratus (= *Hemidactylus leschenaulti*): Holotype, ZSI 5058, from “S.E. Berár, near Chánda” Maharashtra, India.

Hemidactylus cf. *murrayi*: BNHS 1947–1948 (males), BNHS 1949 (female), Aarey Milk Colony, Mumbai, Maharashtra.

Hemidactylus persicus: Holotype, ZSI 5961, from “Persia” (= Iran). The register lists the type as from “Shiraz, Persia.”

Hemidactylus platyceps (= *Hemidactylus gracilis*): Holotype, ZSI 17020, from “Bilimora, Bombay Presidency” Gujarat, India.

Hemidactylus prashadi: BNHS 147 (male), Shirol forest, Belgaum North Kanara, Karnataka, India; BNHS 146 (male), Gersoppa falls, North Kanara, Karnataka, India; ZSI 20123 (female) neighbourhood of Jog, North Kanara district, Bombay Presidency’ (at present in Karnataka, India).

Hemidactylus reticulatus: Type specimens NHM 1874.4.29.410 (male) and NHM 1874.4.29.411 (female), Kollegal, Karnataka, India.

Hemidactylus sataraensis: Holotype BNHS 1743 (female) Chalakewadi, Satara District, Maharashtra, India; paratype BNHS 1742 (female); non-type BNHS 2288 (male), BNHS 2289 (female), Chalakewadi, Satara District, Maharashtra, India.

Hemidactylus scabriceps: Type specimens, ZSI 15353, from “Rámanád,” Tamil Nadu, India.

Hemidactylus sp. (*H.* cf. *maculatus*/*H.* cf. *subtriedrus*): ZSI 24155 (female), Bastar District, Chhattisgarh, India; ZSI 25866 (male) Tyda railway station, Tyda, Vishakapatnam district, Andhra Pradesh, India; ZSI 25708 (male) Ganjam district, Odisha, India; a large male without locality and registration tag along with ZSI 25708.

Hemidactylus sykesii (= *H. maculatus*): Type specimen, NHM XXII.20a (male), Deccan, India (Donated by Indian Museum XXII.20a).

Hemidactylus subtriedroides: Syntype, NHM 1946.8.25.54/ ZSI 4135, “Tsagain, Upper Burma.”

Hemidactylus treutleri: Holotype ZSI 25711 (male), paratype ZSI 25712 (female), outer stone wall of Golconda Fort, Hyderabad, Andhra Pradesh, India.

Hemidactylus triedrus: ZSI 17054 (female) Travindrum, Kerala, India; ZSI 5852, ZSI 5853 (males), Bangalore, Karnataka, India; ZSI 21483, ZSI 21486 (males), Pune, Maharashtra, India.

Appendix I. List of species and their sequence accession numbers for the gene cytochrome *b* used in the present study.

Species	Locality	Accession number
<i>Hemidactylus albofasciatus</i>	Dorle, Ratnagiri, Maharashtra, India	HM595642
<i>Hemidactylus albofasciatus</i>	Malvan, Sindhudurg, Maharashtra, India	HM595643
<i>Hemidactylus</i> cf. <i>gleadowi</i>	Chikkabellapur, Karnataka, India	KU720656
<i>Hemidactylus</i> cf. <i>gleadowi</i>	Ranebennur, Karnataka, India	KU720657
<i>Hemidactylus</i> cf. <i>gleadowi</i>	Mysore, Karnataka, India	KU720658
<i>Hemidactylus</i> cf. <i>gleadowi</i>	Bagalkot, Karnataka, India	KU720659
<i>Hemidactylus</i> cf. <i>gleadowi</i>	Dapoli, Maharashtra, India	KU720660
<i>Hemidactylus</i> cf. <i>gleadowi</i>	Ahmednagar, Maharashtra, India	KU720661
<i>Hemidactylus</i> cf. <i>gleadowi</i>	Iqbalgadh, Gujarat, India	KU720662
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Reasi, Himachal Pradesh, India	KU720648
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Dehradun, Uttarakhand, India	HM595646
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Chamba, Himachal Pradesh, India	KU720649
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Kangra-Jawalamukhi Road, Himachal Pradesh, India	KU720650
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Ajmer, Rajasthan, India	KU720651
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Baripada, Odisha, India	KU720652
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Jammu, India	HM595647
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Chotila, Gujarat, India	KU720653
<i>Hemidactylus</i> cf. <i>kushmorensis</i>	Mt. Abu, Rajasthan, India	KU720654
<i>Hemidactylus</i> cf. <i>murrayi</i>	Badlapur, Maharashtra, India	KU720666
<i>Hemidactylus</i> cf. <i>murrayi</i>	Mumbai, Maharashtra, India	KU720667
<i>Hemidactylus</i> cf. <i>murrayi</i>	Loagan Bunut National Park, Sarawak, Malaysia	GQ375293
<i>Hemidactylus</i> cf. <i>murrayi</i>	Mandalay Division, Myanmar	EU268407
<i>Hemidactylus</i> cf. <i>murrayi</i>	Yangon, Myanmar	GQ375294
<i>Hemidactylus</i> cf. <i>murrayi</i>	Empangon Air Hitam, Pulau Pinang, Malaysia	EU268397
<i>Hemidactylus</i> cf. <i>murrayi</i>	Palakkad, Kerala, India	HM595649
<i>Hemidactylus</i> cf. <i>murrayi</i>	Malshej Ghat, Maharashtra, India	KU720673
<i>Hemidactylus</i> cf. <i>murrayi</i>	Junagadh, Gujarat, India	KU720674
<i>Hemidactylus</i> cf. <i>murrayi</i>	Nasik, Maharashtra, India	KU720676
<i>Hemidactylus frenatus</i>	Sri Lanka, Rathegala	EU268391
<i>Hemidactylus gracilis</i>	Pune, Maharashtra, India	HM595660
<i>Hemidactylus gracilis</i>	Kolhapur, Maharashtra, India	HM595659
<i>Hemidactylus imbricatus</i>	Pakistan (captive specimen)	EU268386
<i>Hemidactylus imbricatus</i>	Pakistan (captive specimen)	EU268385
<i>Hemidactylus parvimaculatus</i>	Gandagan, Odisha, India	KU720637
<i>Hemidactylus parvimaculatus</i>	Polupalli, Tamil Nadu, India	DQ120272
<i>Hemidactylus parvimaculatus</i>	Tumkur, Karnataka, India	HM595645
<i>Hemidactylus parvimaculatus</i>	Mauritius	DQ120271
<i>Hemidactylus parvimaculatus</i>	Mampuri, Sri Lanka	GQ375292
<i>Hemidactylus parvimaculatus</i>	Dehikindagama, Sri Lanka	GQ375296
<i>Hemidactylus parvimaculatus</i>	Matale, Sri Lanka	GQ375298
<i>Hemidactylus parvimaculatus</i>	Gonaganara, Sri Lanka	GQ375297
<i>Hemidactylus parvimaculatus</i>	Kartivu, Sri Lanka	GQ375291
<i>Hemidactylus parvimaculatus</i>	Matale, Sri Lanka	GQ375299
<i>Hemidactylus parvimaculatus</i>	Tempitiya, Sri Lanka	GQ375300

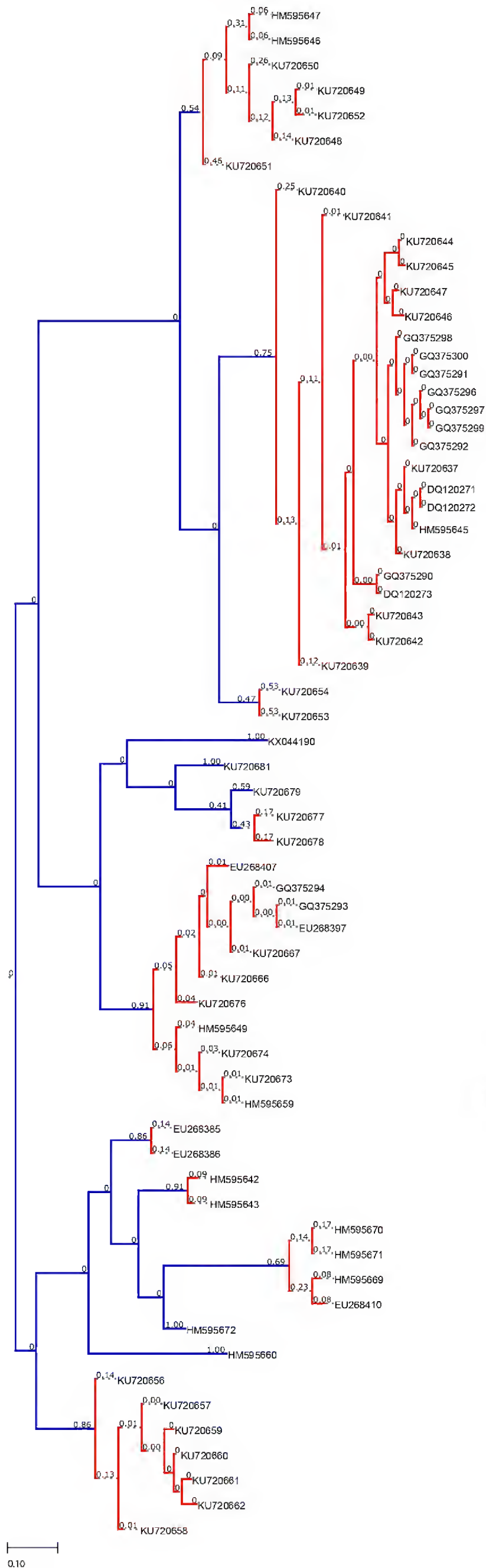
Appendix I (continued). List of species and their sequence accession numbers for the gene cytochrome *b* used in the present study.

Species	Locality	Accession number
<i>Hemidactylus parvimaculatus</i>	Rushikulya, Odisha, India	KU720638
<i>Hemidactylus parvimaculatus</i>	Attagulipura, Karnataka, India	KU720639
<i>Hemidactylus parvimaculatus</i>	Bangalore, Karnataka, India	KU720640
<i>Hemidactylus parvimaculatus</i>	Chennai, Tamil Nadu, India	KU720641
<i>Hemidactylus parvimaculatus</i>	Poinguinim, Goa, India	KU720642
<i>Hemidactylus parvimaculatus</i>	Mollem, Goa, India	KU720643
<i>Hemidactylus parvimaculatus</i>	Kutugam, Odisha, India	KU720644
<i>Hemidactylus parvimaculatus</i>	Araku Valley, Andhra Pradesh, India	KU720645
<i>Hemidactylus parvimaculatus</i>	Vizianagaram, Andhra Pradesh, India	KU720646
<i>Hemidactylus parvimaculatus</i>	Majhiguda, Odisha, India	KU720647
<i>Hemidactylus parvimaculatus</i>	Kollam, Kerala, India	DQ120273
<i>Hemidactylus parvimaculatus</i>	Kandy, Sri Lanka	GQ375290
<i>Hemidactylus reticulatus</i>	Pavgada, Karnataka, India	HM595669
<i>Hemidactylus reticulatus</i>	Nandi Hills, Karnataka, India	HM595670
<i>Hemidactylus reticulatus</i>	Nandi Hills, Karnataka, India	HM595671
<i>Hemidactylus reticulatus</i>	Vellore, Tamil Nadu, India	EU268410
<i>Hemidactylus satarauensis</i>	Chalakewadi, Maharashtra, India	HM595672
<i>Hemidactylus treutleri</i>	Hyderabad, Telangana, India	KU720681
<i>Hemidactylus cf. treutleri</i>	Rishi valley, Andhra Pradesh, India	KU720679
<i>Hemidactylus cf. treutleri</i>	Kangudi, Tamil Nadu, India	KU720678
<i>Hemidactylus cf. treutleri</i>	Chikkabellapur, Karnataka, India	KU720677

Appendix II. PCA loadings for each character

	PC 1	PC 2
SVL	0.2125	0.7975
TRL	0.0613	0.3992
BW	0.0326	0.1582
CL	0.0462	0.1035
TL	0.9720	-0.2202
TW	0.0158	0.1238
HL	0.0123	0.1818
HW	0.0286	0.1637
HH	-0.0010	0.1013
FL	0.0299	0.0967
OD	0.0161	-0.0052
NE	0.0002	0.0720
SE	0.0032	0.0915
EE	0.0032	0.0565
EL	-0.0030	0.0209
IN	-0.0020	0.0209
IO	-0.0276	0.0740

Appendix III. Results of species delimitation using bPTP based on ML tree. Numbers above nodes/tips represent posterior delimitation probabilities from Bayesian reconstruction.



Species 11: *Hemidactylus* cf. *kushmorensis*

Species 9: *Hemidactylus parvimaculatus*

Species 10: *Hemidactylus* cf. *kushmorensis*

Species 1: *Hemidactylus chipkali* sp. nov.

Species 12: *Hemidactylus treutleri* (topotype)

Species 13: *Hemidactylus* cf. *treutleri*

Species 14: *Hemidactylus* cf. *treutleri*

Species 2: *Hemidactylus* cf. *murrayi*

Species 8: *Hemidactylus imbricatus*

Species 7: *Hemidactylus albofasciatus*

Species 5: *Hemidactylus reticulatus*

Species 6: *Hemidactylus satarensis*

Species 3: *Hemidactylus gracilis*

Species 4: *Hemidactylus* cf. *gleadowi*

Appendix IV. Results of bPTP showing support to each species.

bPTP results	Accession numbers	Species
Species 1 (support = 1.000)	KX044190	<i>Hemidactylus chipkali</i> sp. nov.
Species 2 (support = 0.909)	EU268407, GQ375294, GQ375293, EU268397, KU720667, KU720666, KU720676, HM595649, KU720674, KU720673, HM595659	<i>Hemidactylus</i> cf. <i>murrayi</i>
Species 3 (support = 1.000)	HM595660	<i>Hemidactylus gracilis</i>
Species 4 (support = 0.865)	KU720656, KU720657, KU720659, KU720660, KU720661, KU720662, KU720658	<i>Hemidactylus</i> cf. <i>gleadowi</i>
Species 5 (support = 0.689)	HM595670, HM595671, HM595669, EU268410	<i>Hemidactylus reticulatus</i>
Species 6 (support = 1.000)	HM595672	<i>Hemidactylus sataragensis</i>
Species 7 (support = 0.915)	HM595642, HM595643	<i>Hemidactylus albofasciatus</i>
Species 8 (support = 0.860)	EU268385, EU268386	<i>Hemidactylus imbricatus</i>
Species 9 (support = 0.754)	KU720640, KU720641, KU720644, KU720645, KU720647, KU720646, GQ375298, GQ375300, GQ375291, GQ375296, GQ375297, GQ375299, GQ375292, KU720637, DQ120271, DQ120272, HM595645, KU720638, GQ375290, DQ120273, KU720643, KU720642, KU720639	<i>Hemidactylus parvimaculatus</i>
Species 10 (support = 0.469)	KU720654, KU720653	<i>Hemidactylus</i> cf. <i>kushmorensis</i>
Species 11 (support = 0.539)	HM595647, HM595646, KU720650, KU720649, KU720652, KU720648, KU720651	<i>Hemidactylus</i> cf. <i>kushmorensis</i>
Species 12 (support = 1.000)	KU720681	<i>Hemidactylus treutleri</i> (topotype)
Species 13 (support = 0.594)	KU720679	<i>Hemidactylus</i> cf. <i>treutleri</i>
Species 14 (support = 0.425)	KU720677, KU720678	<i>Hemidactylus</i> cf. <i>treutleri</i>

A new gecko of the genus *Hemidactylus*

Appendix V. Un-corrected pairwise sequence divergence between selected species of the genus *Hemidactylus* for the gene cytochrome *b*.

[illegible]

Appendix V (continued). Un-corrected pairwise sequence divergence between selected species of the genus *Hemidactylus* for the gene cytochrome *b*.

27	HM595660	0.17																										
28	HM595669	0.18	0.15																									
29	HM595670	0.17	0.14	0.01																								
30	HM595671	0.17	0.14	0.01	0.00																							
31	HM595672	0.16	0.15	0.11	0.10	0.10																						
32	KU720637	0.16	0.16	0.19	0.18	0.18	0.16																					
33	KU720638	0.15	0.15	0.19	0.18	0.18	0.15	0.02																				
34	KU720639	0.18	0.16	0.20	0.20	0.20	0.18	0.04	0.05																			
35	KU720640	0.18	0.16	0.20	0.20	0.20	0.18	0.04	0.05	0.00																		
36	KU720641	0.18	0.16	0.20	0.20	0.20	0.18	0.03	0.04	0.01	0.01																	
37	KU720642	0.18	0.17	0.21	0.20	0.20	0.18	0.04	0.05	0.04	0.04	0.03																
38	KU720643	0.17	0.16	0.20	0.19	0.19	0.18	0.04	0.05	0.03	0.03	0.03	0.02															
39	KU720644	0.17	0.18	0.20	0.19	0.19	0.18	0.04	0.05	0.05	0.05	0.04	0.05	0.05														
40	KU720645	0.18	0.18	0.20	0.19	0.19	0.18	0.05	0.07	0.05	0.05	0.04	0.05	0.05	0.01													
41	KU720646	0.17	0.17	0.19	0.18	0.18	0.17	0.05	0.07	0.04	0.04	0.04	0.07	0.06	0.04	0.05												
42	KU720647	0.17	0.17	0.19	0.18	0.18	0.17	0.04	0.05	0.04	0.04	0.05	0.05	0.04	0.05	0.06	0.04											
43	KU720648	0.16	0.16	0.18	0.19	0.19	0.18	0.12	0.11	0.11	0.11	0.11	0.14	0.12	0.13	0.13	0.13	0.13										
44	KU720649	0.16	0.16	0.18	0.19	0.19	0.18	0.13	0.12	0.12	0.12	0.12	0.14	0.13	0.14	0.14	0.13	0.13	0.01									
45	KU720650	0.16	0.16	0.18	0.19	0.19	0.18	0.12	0.11	0.11	0.11	0.11	0.14	0.12	0.13	0.13	0.13	0.13	0.00	0.01								
46	KU720651	0.15	0.15	0.18	0.18	0.18	0.17	0.11	0.11	0.11	0.11	0.11	0.13	0.11	0.13	0.13	0.12	0.12	0.01	0.01	0.01							
47	KU720652	0.16	0.16	0.19	0.20	0.20	0.18	0.11	0.11	0.11	0.11	0.11	0.13	0.11	0.13	0.14	0.12	0.12	0.02	0.02	0.02	0.02						
48	KU720653	0.14	0.16	0.21	0.20	0.20	0.16	0.11	0.10	0.11	0.11	0.11	0.14	0.12	0.13	0.14	0.13	0.13	0.10	0.10	0.10	0.09	0.09					
49	KU720654	0.14	0.16	0.21	0.20	0.20	0.16	0.11	0.10	0.11	0.11	0.11	0.14	0.12	0.13	0.14	0.13	0.13	0.10	0.10	0.10	0.09	0.09	0.00				
50	KU720656	0.14	0.15	0.15	0.15	0.15	0.12	0.15	0.14	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.16	0.14	0.14	0.14	0.14	0.15	0.15	0.15			
51	KU720657	0.14	0.16	0.15	0.15	0.15	0.11	0.15	0.14	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.16	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.01		
52	KU720658	0.13	0.15	0.15	0.15	0.15	0.12	0.15	0.13	0.15	0.15	0.15	0.16	0.15	0.14	0.15	0.14	0.15	0.14	0.14	0.14	0.13	0.14	0.14	0.14	0.01		
53	KU720659	0.13	0.16	0.16	0.16	0.16	0.12	0.16	0.14	0.16	0.16	0.16	0.17	0.16	0.15	0.16	0.15	0.16	0.15	0.15	0.15	0.14	0.15	0.15	0.15	0.02		
54	KU720660	0.14	0.16	0.16	0.16	0.16	0.13	0.16	0.15	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.02		
55	KU720661	0.14	0.15	0.16	0.16	0.16	0.12	0.15	0.13	0.16	0.16	0.16	0.18	0.17	0.16	0.17	0.16	0.17	0.15	0.15	0.15	0.14	0.15	0.15	0.15	0.03		
56	KU720662	0.13	0.15	0.16	0.15	0.15	0.13	0.15	0.15	0.17	0.17	0.17	0.18	0.17	0.16	0.16	0.17	0.18	0.16	0.16	0.16	0.16	0.17	0.15	0.15	0.05		
57	KU720666	0.02	0.18	0.20	0.19	0.19	0.17	0.18	0.17	0.20	0.20	0.20	0.21	0.20	0.18	0.19	0.20	0.19	0.17	0.17	0.17	0.16	0.17	0.15	0.15	0.15		
52	KU720658	0.01																										
53	KU720659	0.01	0.02																									
54	KU720660	0.01	0.02	0.01																								
55	KU720661	0.03	0.03	0.04	0.03																							
56	KU720662	0.04	0.05	0.04	0.04	0.04																						
57	KU720666	0.15	0.14	0.14	0.15	0.15	0.14																					

Appendix V (continued). Un-corrected pairwise sequence divergence between selected species of the genus *Hemidactylus* for the gene cytochrome *b*.

58	KU720667	0.17	0.17	0.16	0.17	0.17	0.16	0.00	0.02	0.20	0.16	0.16	0.16	0.00	0.00	0.16	0.16	0.17	0.16	0.16	0.18	0.18	0.17	0.15	0.16	0.03	
59	KU720673	0.16	0.16	0.16	0.16	0.17	0.16	0.03	0.04	0.18	0.15	0.16	0.16	0.03	0.03	0.16	0.16	0.16	0.16	0.16	0.19	0.19	0.16	0.16	0.16	0.00	
60	KU720674	0.16	0.16	0.16	0.16	0.17	0.16	0.03	0.04	0.18	0.15	0.16	0.16	0.03	0.03	0.16	0.16	0.16	0.16	0.16	0.19	0.19	0.16	0.16	0.16	0.00	
61	KU720676	0.16	0.16	0.16	0.16	0.16	0.15	0.07	0.08	0.18	0.16	0.15	0.15	0.07	0.07	0.15	0.15	0.16	0.15	0.15	0.20	0.20	0.16	0.14	0.15	0.06	
62	KU720677	0.17	0.17	0.16	0.14	0.15	0.17	0.11	0.13	0.17	0.15	0.16	0.16	0.11	0.11	0.16	0.16	0.17	0.16	0.16	0.18	0.18	0.17	0.16	0.16	0.12	
63	KU720678	0.16	0.16	0.17	0.15	0.16	0.18	0.13	0.14	0.17	0.17	0.16	0.16	0.13	0.13	0.16	0.16	0.16	0.16	0.16	0.18	0.18	0.16	0.16	0.16	0.13	
64	KU720679	0.17	0.17	0.17	0.15	0.15	0.15	0.11	0.13	0.16	0.17	0.17	0.17	0.11	0.11	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.15	0.16	0.10	
65	KU720681	0.16	0.16	0.17	0.18	0.18	0.15	0.12	0.14	0.15	0.17	0.15	0.15	0.12	0.12	0.15	0.15	0.16	0.15	0.15	0.19	0.19	0.16	0.16	0.16	0.13	
66	KX044190	0.22	0.22	0.21	0.17	0.18	0.17	0.13	0.14	0.20	0.20	0.22	0.22	0.13	0.13	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.18	0.18	0.11	
58	KU720667	0.03	0.17	0.20	0.19	0.19	0.16	0.16	0.16	0.18	0.18	0.18	0.19	0.18	0.17	0.17	0.18	0.17	0.15	0.16	0.15	0.15	0.16	0.14	0.14	0.14	
59	KU720673	0.00	0.17	0.18	0.17	0.17	0.16	0.16	0.15	0.18	0.18	0.18	0.18	0.17	0.17	0.18	0.17	0.17	0.16	0.16	0.16	0.15	0.16	0.14	0.14	0.14	
60	KU720674	0.00	0.17	0.18	0.17	0.17	0.16	0.16	0.15	0.18	0.18	0.18	0.18	0.17	0.17	0.18	0.17	0.17	0.16	0.16	0.16	0.15	0.16	0.14	0.14	0.14	
61	KU720676	0.06	0.16	0.18	0.17	0.17	0.17	0.15	0.15	0.17	0.17	0.17	0.18	0.17	0.16	0.16	0.18	0.16	0.14	0.15	0.14	0.14	0.15	0.15	0.15	0.13	
62	KU720677	0.12	0.17	0.17	0.16	0.16	0.14	0.16	0.17	0.17	0.17	0.17	0.19	0.18	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.15	0.16	0.14	0.14	0.13	
63	KU720678	0.13	0.18	0.17	0.16	0.16	0.13	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.16	0.17	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.14	
64	KU720679	0.10	0.18	0.16	0.16	0.16	0.14	0.17	0.17	0.18	0.18	0.18	0.20	0.18	0.17	0.18	0.17	0.18	0.15	0.16	0.15	0.15	0.15	0.14	0.14	0.12	
65	KU720681	0.13	0.17	0.16	0.16	0.16	0.16	0.15	0.16	0.15	0.15	0.15	0.17	0.17	0.16	0.15	0.16	0.16	0.16	0.16	0.16	0.15	0.17	0.16	0.16	0.14	
66	KX044190	0.11	0.19	0.20	0.20	0.20	0.21	0.22	0.21	0.22	0.22	0.22	0.23	0.22	0.22	0.23	0.22	0.22	0.18	0.18	0.18	0.17	0.18	0.16	0.16	0.16	
58	KU720667	0.13	0.13	0.12	0.13	0.14	0.13	0.02																			
59	KU720673	0.14	0.13	0.13	0.14	0.14	0.13	0.02	0.03																		
60	KU720674	0.14	0.13	0.13	0.14	0.14	0.13	0.02	0.03	0.00																	
61	KU720676	0.13	0.12	0.14	0.14	0.13	0.13	0.06	0.07	0.06	0.06																
62	KU720677	0.13	0.13	0.13	0.14	0.13	0.14	0.13	0.11	0.12	0.12	0.14															
63	KU720678	0.14	0.14	0.14	0.15	0.14	0.15	0.14	0.13	0.13	0.13	0.15	0.03														
64	KU720679	0.12	0.13	0.12	0.13	0.11	0.13	0.11	0.11	0.10	0.10	0.12	0.04	0.05													
65	KU720681	0.14	0.13	0.13	0.14	0.14	0.14	0.14	0.12	0.13	0.13	0.13	0.10	0.09	0.10												
66	KX044190	0.17	0.16	0.16	0.16	0.16	0.16	0.11	0.13	0.11	0.11	0.14	0.15	0.17	0.14	0.16											



Larval development and breeding ecology of Ziegler's Crocodile Newt, *Tylototriton ziegleri* Nishikawa, Matsui and Nguyen, 2013 (Caudata: Salamandridae), compared to other *Tylototriton* representatives

^{1,2}Marta Bernardes, ¹Anna Rauhaus, ²Clara Michel, ^{3,8}Cuong The Pham, ^{3,8}Truong Quang Nguyen, ^{4,5,6}Minh Duc Le, ⁷Frank Pasmans, ²Michael Bonkowski, and ^{1,2,*}Thomas Ziegler

¹Cologne Zoo, Riehler Straße 173, 50735, Cologne, GERMANY ²Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Zùlpicher Straße 47b, 50674, Cologne, GERMANY ³Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Hanoi, VIETNAM ⁴Faculty of Environmental Sciences, Hanoi University of Science, Vietnam National University, 334 Nguyen Trai Road, Hanoi, VIETNAM ⁵Central Institute for Natural Resources and Environmental Studies, Hanoi National University, 19 Le Thanh Tong, Hanoi, VIETNAM ⁶Department of Herpetology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024 ⁷Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B 9820 Merelbeke, BELGIUM ⁸Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.

Abstract.—We describe for the first time the larval development and stages of the recently described Ziegler's Crocodile Newt (*Tylototriton ziegleri*), an endemic species to northern Vietnam. Diagnostic morphological characters are provided for Grosse (1997, 2013) stages 27–32, 35–36, and 44–45, as well as comparisons with larval stages of other *Tylototriton* representatives. In addition, natural history data and an ecological assessment of the breeding niche are presented for *T. ziegleri* as well as for *T. vietnamensis*, from whom the former species was only recently taxonomically separated. We provide data extending the known breeding season of these two cryptic species in the North of Vietnam, which in fact lasts from April until July. On average, the clutches of *T. ziegleri* consisted of 67 ± 32 eggs, were found on rock and soil substrates with a distance of 50 ± 28 cm from water, whereas the clutches of *T. vietnamensis* were significantly smaller (43 ± 19 eggs), found only on soil and were further distant from water (80 ± 41 cm). The known maximum altitudinal distribution of *T. vietnamensis* is herein increased to 980 m above sea level. Based on the examples of *T. ziegleri* and *T. vietnamensis*, this study highlights how important it is to uncover cryptic species, define their exact distribution range, and investigate potential differences in ecological adaptations in order to assess the conservation status, develop proper conservation planning and provide suitable conditions for potential *ex situ* breeding programs.

Keywords. Vietnam, Crocodile Newts, cryptic species, developmental biology, larval staging, microhabitat characterization, conservation, captive breeding

Citation: Bernardes M, Rauhaus A, Michel C, Pham CT, Nguyen TQ, Le MD, Pasmans F, Bonkowski M, Ziegler T. 2017. Larval development and breeding ecology of Ziegler's Crocodile Newt, *Tylototriton ziegleri* Nishikawa, Matsui and Nguyen, 2013 (Caudata: Salamandridae), compared to other *Tylototriton* representatives. *Amphibian & Reptile Conservation* 11(1) [General Section]: 72–87 (e138).

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Received: 04 Nov 2016; **Accepted:** 02 March 2017; **Published:** 17 April 2017

Introduction

The genus *Tylototriton* currently consists of 22 species with a distribution from Nepal, Bhutan, and India eastwards to China and southwards to Indochina (Nishikawa et al. 2013a). Phylogenetic analyses divided the genus into the *T. asperrimus* group (Fei et al. 2005) or the subgenus *Yaotriton* (Dubois and Raffaelli 2009), which

includes: *T. asperrimus*, *T. broadoridgus*, *T. dabienicus*, *T. hainanensis*, *T. liuyangensis*, *T. lizhenchangi*, *T. notialis*, *T. podichthys*, *T. panhai*, *T. vietnamensis*, *T. wenxiensis*, and *T. ziegleri*; and the *T. verrucosus* group (Fei et al. 2005) or the subgenus *Tylototriton* (Dubois and Raffaelli 2009), which includes: *T. anguliceps*, *T. himalayanus*, *T. kweichowensis*, *T. pseudoverrucosus*, *T. shanjing*, *T. shanorum*, *T. taliangensis*, *T. uyenoi*, *T. verrucosus*.

Correspondence. Email: *ziegler@koelnerzoo.de



Fig. 1. A. Adult male of *Tylototriton ziegleri*; B. Habitat type in Bao Lac district, Cao Bang Province; C. Adult male of *Tylototriton vietnamensis*; D. Habitat type in Tay Yen Tu Nature Reserve, Bac Giang Province. Photos M. Bernardes.

sus, and *T. yangi* (Khatiwada et al. 2015; Le et al. 2015; Nishikawa et al. 2014; Phimmachak et al. 2015; Yang et al. 2014).

In Vietnam, this genus is currently represented by *T. asperrimus*, *T. notialis*, *T. anguliceps*, as well as by two endemic species, viz. *T. vietnamensis*, and *T. ziegleri*. Specimens of Ziegler's Crocodile Newt were previously referred to *T. asperrimus* (Sparreboom et al. 2011, Yuan et al. 2011) or *T. cf. vietnamensis* (Stuart et al. 2010). *Tylototriton ziegleri* was subsequently described as a distinct species by Nishikawa et al. (2013b) based on morphological and molecular differences from *T. vietnamensis*. The latter species has been evaluated as Endangered in the Vietnam Red Data Book (Tran et al. 2007), and in the IUCN Red List (IUCN SSC Amphibian Specialist Group 2016).

Tylototriton vietnamensis inhabits secondary evergreen lowland forests on granite parent rock material consisting of hardwood, bamboo and shrubs and is known from lower elevations in Bac Giang, Quang Ninh, Lang Son, and Phu Tho provinces (Bernardes et al. 2017; Nguyen et al. 2009). In contrast, *T. ziegleri* is known from primary forests on limestone parent rock material at higher elevations characterized mainly by bamboo vegetation in Cao Bang and Ha Giang provinces (Nishikawa et al. 2013b) (Fig. 1).

In-depth studies focusing on distinctive features and thus on the taxonomic status of closely related or at least similar, potential taxa which are either threatened and/

or have a limited range, as in the case of the species pair *T. vietnamensis* and *T. ziegleri*, are important for proper identification and suitable conservation actions. Since both species are distributed at different elevations and occupy distinct geological areas, we expected to find ecological, morphological, and developmental differences to support their discrimination. Herein, we document for the first time the larval development of *T. ziegleri*, in comparison with information on the development of other *Tylototriton* species. We also provide data on the ecological niche of *T. ziegleri*, in particular microhabitat preferences associated with reproduction, and compare this with our own field data for *T. vietnamensis*.

Materials and Methods

Field surveys. Field surveys were conducted by M. Bernardes, C.T. Pham and H.T. An during the rainy season between 10 April and 11 July 2010, 8 June and 7 August 2012, 13 June and 28 July 2013, and 15 May and 28 June 2014 in northern Vietnam. The surveys were conducted in Son Dong and Luc Nam districts in Bac Giang Province, Hoanh Bo district in Quang Ninh Province, and Mau Son district in Lang Son Province for *T. vietnamensis*; and in Quan Ba and Bac Quang districts in Ha Giang Province, and Bao Lac district in Cao Bang Province for *T. ziegleri*. Daytime visits to breeding sites were conducted for an average time of 20 minutes and varied between two and eight times, sometimes in repeated

years. Besides coordinates and elevations recorded with Garmin GPS MAP62, a defined set of several abiotic variables were collected and recorded in order to classify each study site according to their environmental conditions. The physical characteristics of each pond (area and maximum depth) were determined by use of a measuring rope of precisely known size. A pH meter (Hanna HI 98129) calibrated for 25 °C was used to measure the pH and record water temperature. Water chemical parameters were taken for an analysis of pollution (concentration of nitrate [NO₃-] and nitrite [NO₂-]) and water hardness (carbonate [KH] and total hardness [gH], measurements following the German degree) using drop-by-drop color tests from JBL (Testlab, Germany). Since some pools occurred only temporary the hydroperiod was also taken into account. Weather data consisting of temperature, humidity, and atmospheric pressure were recorded with a weather station (Krestel 3500) at each breeding site. The tree canopy cover above the pond water was assessed visually and classified in five levels ranging from 0–100% cover.

Adult animals were searched on the bottom of the water body using visual survey encounter techniques followed by subsequent capture with a sweep of a 25 cm dip-net (JBL, Germany). The surrounding shore of the pond was searched for the presence of egg clutches, efts, and additional adults until a maximum distance of three meters from the water line. All captured adults were counted, photographed, and subsequently released. The distance of the egg clutch to the water source was measured and the total number of eggs present in the clutches counted with minimum disturbance. The diameter and the weight of seven randomly picked eggs (in the case of *T. ziegleri* belonging only to Bao Lac district, Cao Bang Province) were measured by using a digital calliper to the nearest 0.1 mm and weighted with a digital scale to the nearest 0.01 g.

Larval staging, morphological description and comparisons with congeners. One clutch of *T. ziegleri* was collected on the 17th July 2014 in Bao Lac district, Cao Bang Province, Vietnam to observe the larval development and for further morphological comparisons. The clutch was collected together with associated substrate and foliage coverage from the shore of a breeding pond. The clutch was kept inside an open-air plastic box and regularly sprayed with water to keep up the suitable humidity level. Due to the thickness of the gelatinous layer (albumen) it was not possible to observe and document the developing larvae inside; therefore we preserved few eggs for morphological analysis. Every one or two days one egg was randomly selected (in total 23), transferred to a 4% formalin solution for fixation and subsequently preserved in 70% ethanol. This procedure was repeated until hatching of the first larva. The remaining larvae were later transferred to the Me Linh Station for Biodiversity to contribute to a captive breeding pro-

gram. In addition, seven swimming larvae were collected at the breeding pond for a morphological description of more advanced developing stages. The larvae were anaesthetized with ethyl acetate, fixed in 40% ethanol, and later transferred to 70% ethanol for preservation. Preserved individuals subsequently were deposited in the collections of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam, with the catalogue numbers: IEBR A.2016.19-A.2016.31 and of the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany, with the catalogue numbers: ZFMK 98792-ZFMK 98796.

Preserved eggs were cut open to examine the developing larvae inside. A detailed description of the development and measurements of the ovum in early stages could not be performed as the jelly layers could not be opened without destroying the fragile content. Staging was performed under a magnifying loupe and by support from a digital microscope (Keyence VHX-500F) when extra magnification, photographs, and morphological measurements were needed. To complement the characterization process (e.g., for characterizing the coloration in life), additional photographs of hatched larvae were taken during field work by M. Bernardes or at the Me Linh Station for Biodiversity by T. Ziegler by placing single larvae into a water filled glass vessel.

Larval stages were identified according to Grosse (1997, 2013) and the morphological terminology followed Nishikawa et al. (2013b). The following measurements were taken: snout-vent length (SVL), from tip of snout to posterior edge of the vent; head length (HL), from posterior edge of right parotid to snout tip; maximum head width (HW); head height (HH), measured above the eyes; snout length (SL), from right nostril to right posterior corner of mouth; interocular distance (IoD), from anterior corner of eyes; internostril distance (InD); eye-nostril distance (EnD), from right nostril to anterior corner of right eye; forelimb length (FIL), from right anterior limb measured from point of body insertion to tip of longest finger; hind-limb length (HIL), right posterior limb measured from point of body insertion to tip of longest toe; axillar distance (AD), from axilla to groin on right side; width of tail base (TW), measured at posterior edge of vent; maximum tail height (TH); tail length (TaL), from posterior edge of vent to tail tip; total length (TL), from tip of snout to tail tip.

For morphological comparisons, data from literature was included for the following species: *T. anguliceps*, *T. asperimus*, *T. broadoridgus*, *T. hainanensis*, *T. himalayanus*, *T. kweichowensis*, *T. liuyangensis*, *T. podichthys*, *T. cf. shanjing*, *T. shanorum*, *T. taliangensis*, *T. uyenoi*, and *T. wenxianensis* (see Bourret 1942; Khatiwada et al. 2015; Kuzmin et al. 1994; Mudrack 2005; Nishikawa et al. 2013a, 2014, 2015; Phimmachak et al. 2015; Shen et al. 2012; Sparreboom 2014; Yang et al. 2014; Zhao 1988; Ziegler et al. 2008). For detailed comparisons with *T. vietnamensis* we included our own field data and pictures

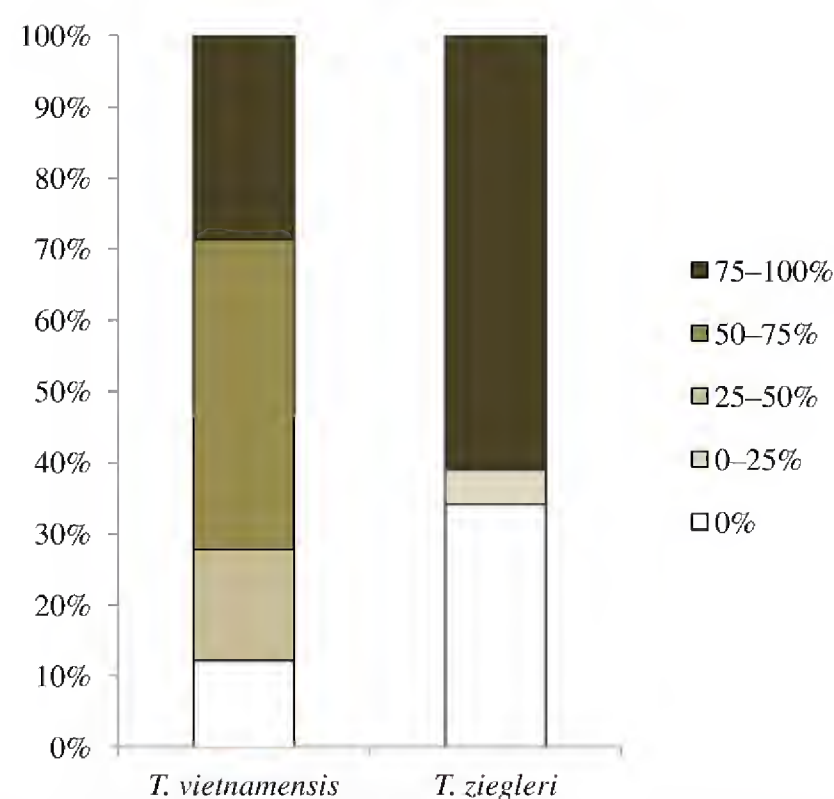


Fig. 2. Percentage of the number of adults of *Tylototriton vietnamensis* and *T. ziegleri* found at each interval of percentage of canopy cover measured above the water of the breeding site.

of larvae (in stages 33, 35, 41, and 42) photographed either *in situ* during our field work in Bac Giang Province by M. Bernardes or *ex situ* at the Me Linh Station for Biodiversity by T. Ziegler.

Statistical analysis. Comparisons between *T. ziegleri* and *T. vietnamensis* regarding the area and depth of the different ponds, clutch sizes, and distance to water, as well as the regression between the clutch and egg sizes within the genus were examined with Student’s *t*-test after confirming a normal distribution of the data. Analyses were performed in R version 3.2.3.

Molecular analysis. For species identification, we sequenced a partial mitochondrial gene, the NADH dehydrogenase subunit 2 (ND2), for the egg / larval tissue samples (IEBR A.2016.19–A.2016.21) from the clutch of *T. ziegleri* collected on the 17th July 2014 in Bao Lac district, Cao Bang Province, Vietnam, which was used for larval staging, using the primer pair, Sal_ND2_F1

and Sal_ND2_R2 (Nishikawa et al. 2013b). Tissue samples were extracted using DNeasy blood and tissue kit, Qiagen (California, USA). Extracted DNA from the fresh tissue was amplified by PCR mastermix (Fermentas, Canada). The PCR volume consisted of 21 µl (10 µl of mastermix, five µl of water, two µl of each primer at 10 pmol/µl, and two µl of DNA or higher depending on the quantity of DNA in the final extraction solution). PCR condition was: 95 °C for five minutes to activate the taq; with 40 cycles at 95 °C for 30 s, 50 °C for 45 s, 72 °C for 60 s; and the final extension at 72 °C for six minutes.

PCR products were subjected to electrophoresis through a 1% agarose gel (UltraPure™, Invitrogen). Gels were stained for 10 minutes in 1X TBE buffer at two pg/ml of ethidium-bromide, and visualized under UV light. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purification kit (Fermentas, Canada). Purified PCR products were sent to Macrogen Inc. (Seoul, South Korea) for sequencing.

Sequences generated in this study were aligned with one another using the De Novo Assemble function in the program Geneious v.7.1.8 (Kearse et al. 2012). They were then compared with other sequences using the Basic Local Alignment Search Tool (BLAST) in GenBank.

Results

Molecular analysis. Three sequences of 987 bps were obtained. The sequences were almost identical, except in two positions, and 99% to 100% similar to the sequence with the GenBank’s accession number AB769542 of *T. ziegleri* (voucher VNMN 3389). The results confirm the samples collected in Cao Bang Province are conspecific with *T. ziegleri*.

Distribution, ecological niche and microhabitat use of *T. ziegleri* and *T. vietnamensis*. *Tylototriton ziegleri* was found in Cao Bang Province at elevations between 1,325 and 1,420 m above sea level, in Ha Giang Province, Bac Quang district between 868 and 932 m above sea level, and in Quan Ba district between 1,080

Table 1. Results of water chemical analysis conducted during field work in the habitat of *Tylototriton vietnamensis* and *T. ziegleri* during 2010, 2013, and 2014. Values are presented as min. – max. (mean ± standard deviation).

Species	Province	district	pH	°KH	°gH	NO ₂ ⁻ (mg l ⁻¹)	NO ₃ ⁻ (mg l ⁻¹)
<i>T. vietnamensis</i>	Bac Giang	Son Dong	4.65–6.43	1–5	1–5.5	0–0.5	0–10
			(5.48 ± 0.48)	(1.80 ± 1.11)	(2.46 ± 1.63)	(0.04 ± 0.11)	(3.39 ± 2.38)
	Quang Ninh	Uong Bi	7.36–7.51	4–8	5–6	0–0.4	5–15
			(7.43 ± 0.11)	(6 ± 2.83)	(5.5 ± 0.71)	(0.2 ± 0.28)	(10 ± 7.07)
<i>T. ziegleri</i>	Cao Bang	Bao Lac	7.08–7.28	6–6	7–7	0.03–0.03	0.03–20
			(7.18 ± 0.14)			(10.01 ± 14.12)	
	Ha Giang	Bac Quang	6.36–7.05	1–1	1–2	0–0.05	1–1
			(6.63 ± 0.37)		(1.33 ± 0.58)	(0.03 ± 0.03)	
	Ha Giang	Quan Ba	6.41–7.94	1–8	1–9	0.05–0.4	0–1
			(7.30 ± 0.61)	(5.33 ± 2.88)	(5 ± 3.41)	(0.13 ± 0.14)	(0.79 ± 0.4)



Fig. 3. **A:** Typical clutch of *Tylototriton ziegleri* composed by single eggs; **B:** an exceptional case of “stickiness” where eggs were aggregated in groups of 2–4. Photos M. Bernardes.

and 1,369 m above sea level. *Tylototriton vietnamensis* was found between 181 and 512 m above sea level in Bac Giang and Quang Ninh provinces, and between 840 and 980 m above sea level in Lang Son Province. Spawning sites consisted of small ponds for both species, although in the district Quan Ba, Ha Giang Province we also found clutches of *T. ziegleri* in the slopes of a slow flowing forest stream, suggesting that this species can also breed in this type of habitat. A physical evaluation of ponds during our field work showed that the ones inhabited by *T. ziegleri* were significantly deeper ($F_{1,42} = 25.11$, $P < 0.001$; mean 79 ± 58 cm, $n = 19$, range between 10 and 200 cm) than those inhabited by *T. vietnamensis* (mean 25 ± 14 cm, $n = 81$, range between 3 and 60 cm), while the area was roughly the same ($F_{1,44} = 0.004$, $P = 0.95$; *T. ziegleri*: mean 84 ± 165 m², range between 2.5 and 510 m²; *T. vietnamensis*: mean 82 ± 102 m², range between one and 460 m²). Most adults (61% of 82 individuals of *T. ziegleri* and 72.2% of 255 individuals of *T. vietnamensis*) were found in breeding sites with 50% or more canopy cover, although still 34.1% of all *T. ziegleri* and 12.2% of all *T. vietnamensis* were found in breeding sites with no canopy cover (Fig. 2).

A comparison of water quality showed that *T. ziegleri* occurred in ponds with pH values between 6.4 and 8 (mean 7 ± 0.5 ; throughout Cao Bang and Ha Giang provinces), while *T. vietnamensis* occurred in ponds with pH values ranging from 4.7 to 7.5 (mean 5.6 ± 0.7 ; through-



Fig. 4. Drawing of a formol-preserved larva of *Tylototriton ziegleri* at stage 35. Drawing C. Michel.

out Bac Giang and Quang Ninh provinces). Following US Geological Survey standard for water hardness classification both *T. ziegleri* and *T. vietnamensis* varied between soft (0–1 °KH) and hard (8 °KH), with *T. ziegleri* distributed over an average of 4 ± 3 °KH and *T. vietnamensis* over an average of 2 ± 2 °KH. The general hardness was also on average higher for *T. ziegleri* (1–9 °gH; mean 4 ± 3 °gH) compared to *T. vietnamensis* (1–6 °gH; mean 3 ± 2 °gH). Concentration of nitrite ranged from 0–0.4 mg l⁻¹ for *T. ziegleri* and from 0–0.5 mg l⁻¹ for *T. vietnamensis*, while concentrations of nitrate ranged from 0–20 mg l⁻¹ and 0–15 mg l⁻¹, respectively (Table 1). Environmental data revealed higher humidity levels for *T. ziegleri* (mean $100 \pm 0\%$) than those for *T. vietnamensis* (mean $94 \pm 9\%$, range between 68 and 100%), and slightly higher temperature oscillations for *T. vietnamensis* 24.2–34.2 °C (mean 28.6 ± 2.2 °C) than those for *T. ziegleri* 26–34.4 °C (mean 27.4 ± 3.3 °C).

Mating and egg deposition of *T. ziegleri* and *T. vietnamensis*.

During the breeding season of these two species (April–July) reproductive males that were otherwise terrestrial, moved into the water at the breeding sites and waited for the females. When precipitation was lacking and breeding sites dried out, adults were forced to maintain their terrestrial life mode. However, if climatic conditions were favorable, males preferably were found inside the water. From a total of 547 captured adults of *T. vietnamensis*, and 101 adults of *T. ziegleri*, only five (0.91%) and two (1.98%), respectively, were found on land. Females seem to join the males in the water for a very short period, since only 12 females of *T. vietnamensis* and one female of *T. ziegleri* were captured during field work. After courtship and reproduction, gravid females laid large eggs at the shore of the water body and returned to the forest. Due to their slight stickiness, the eggs aggregated in egg masses (Fig. 3A) which were subsequently covered by the females with leaf litter from the forest ground. The clutch size was significantly different for the two species ($F_{1,71} = 11.29$, $P < 0.001$). For *T. ziegleri*, it ranged between 10 and 109 eggs (mean 67 ± 32 eggs, $n = 10$), with clutches found between rock (83.3%) and soil (16.7%) substrates, while for *T. vietnamensis* ($n = 63$) it ranged between 5 and 85 eggs (mean 43 ± 19 eggs) and clutches were always found on soil substrate. Clutches of *T. ziegleri* and *T. vietnamensis* differed significantly in their distances to the nearest water



Fig. 5. Metamorph of *Tylostotriton ziegleri* at stage 44, with an additional finger on left hand, collected in Ha Giang Province in 2012 and preserved in ethanol. Photo M. Bernardes.

body ($F_{1,50} = 5.32$, $P < 0.01$). Clutches of *T. ziegleri* were between 10 and 100 cm away from water (mean 50 ± 28 cm, $n = 11$), while the ones from *T. vietnamensis* were found at a distance between 17 and 188 cm (mean 80 ± 41 cm, $n = 41$) from the water.

In one exceptional case a clutch of *T. ziegleri* consisted of eggs sticking so strongly together that they were no longer solitary but formed small aggregations of two to four eggs (Fig. 3B).

Egg description, developmental stages and larval morphology of *T. ziegleri*. From a total of 80 eggs in the collected clutch of *T. ziegleri*, 34 (42%) did

not show normal development. The same count was done in the field with one other clutch consisting of 107 eggs and revealed that 23% of the eggs had ceased to develop.

We estimated that the collected egg clutch was around two days old at the time of collection (16th June 2014). The diameter of the preserved eggs ranged between 8.7 and 11.2 mm (mean 10.1 ± 0.8 mm, $n = 23$), while the diameter of eggs measured randomly in the field (all measurements of the eggs from Bao Lac district, Cao Bang Province) ranged between 7.2 and 8.9 mm (mean 8.1 ± 0.8 mm, $n = 28$). The associated weight for the eggs measured in the field ranged between 0.2 and 0.4 g (mean 0.3 ± 0.1 g, $n = 28$).

In general, the egg shape was round and the surface of the jelly layers uneven. Most eggs contained a clear gelatinous layer, few were slightly more opaque. The liquid inside was clear. The capsular chamber contained the embryo or in less developed stages the ovum which was either attached or not attached to the inside of the chamber. An outer observation of the ovum in early stages identified a round ovum with a creamy yellowish white color. The size of three measured ovae varied between 3.87 and 4.78 mm. Larvae in an advanced stage of development showed a more slender shape, curved to fit inside the capsular chamber. While some stages were represented by multiple specimens, stages 33, 34, and 37–43 were not found and thus could not be examined. At stage 27 (IEBR.A.2016.22) gill and forelimb buds were discernible. Stage 28 (IEBR.A.2016.23) was characterized by further development of the forelimb buds.

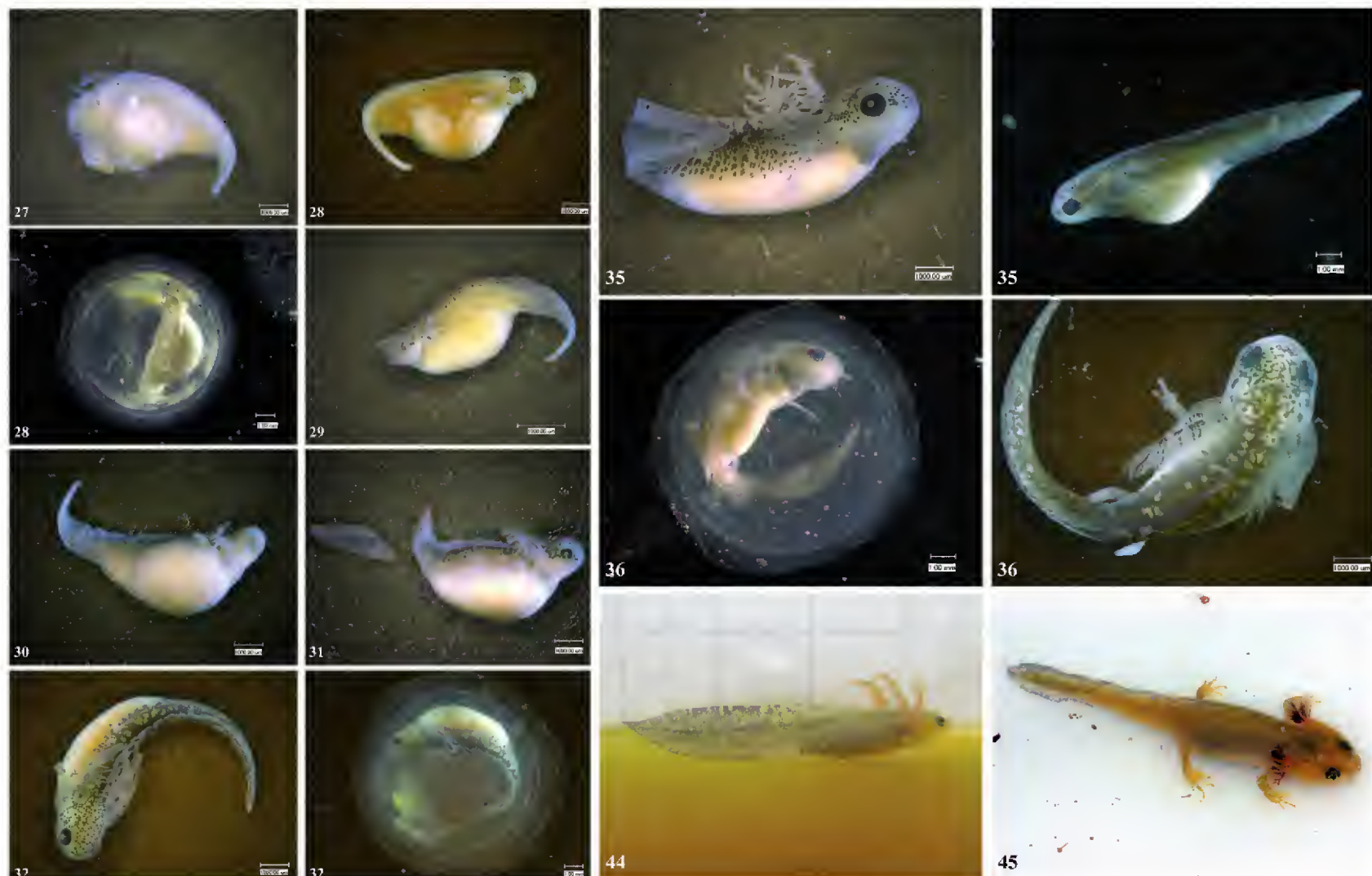


Fig. 6. *Tylostotriton ziegleri* larvae from Bao Lac district, Cao Bang Province with indication of the corresponding developmental stage and scale. Photographs of stages 27 to 36 are from preserved eggs photographed under a digital microscope (photos C. Michel) and photographs of stages 44 and 45 are from individuals in life kept at the Me Linh station (photos T. Ziegler).

This was also the case for stage 29 (IEBR.A.2016.24 and ZFMK 98792) along with the growth of fimbriae. Stage 30 (IEBR.A.2016.25) could be determined by the dome shaped distal tip of the forelimb. The forelimb was cone shaped at stage 31 (IEBR.A.2016.26 and ZFMK 98793). Two digits were formed at stage 32 (IEBR.A.2016.27). At stage 35 (IEBR.A.2016.28 and ZFMK 98794) the forelimb had a joint and a hand with three digits. Additionally the hind limbs started to develop and in some cases already showed toe buds (see Fig. 4). At this stage, around 20 days after the assumed egg deposition date (4th July), the first larvae hatched, while some still remained

inside the egg. At hatching time larvae had an average total size of 14.65 ± 0.77 mm (size ranged between 13.78 and 15.22 mm, $n = 3$). Stage 36 (IEBR.A.2016.29 and ZFMK 98795) was characterized by a forelimb with four digits and a hind limb with three toes and a knee joint. The yolk sac was prominent in stages 27–30 and was evident until stage 35. At stages 44 (IEBR.A.2016.30) and 45 (IEBR.A.2016.31 and ZFMK 98796), larvae were black and had well developed limbs with four fingers and five toes, and the gills atrophied. In one individual at stage 44 we observed the splitting of one finger in two, resulting in five fingers on the left hand (Fig. 5). No juve-

Table 2. Developmental stages, morphological description and coloration of *Tylototriton ziegleri* from stages 27 to 45; stage diagnostic characteristics according to Grosse (1997, 2013) are italicized. Specimens from stages 27–36 originated from the egg clutch while data on stages 44 and 45 were gathered from hatched larvae collected inside the breeding pond.

Stage	Morphology	Coloration
27 ($n = 1$)	Head trapezoidal and sloping in profile, snout short and flat, no labial fold visible, eyes distinguishable but unobtrusive. Gills upright, shorter than head. No balancers. Dorsal and ventral fins about the same height, higher than head; dorsal fin starts at last third of the trunk; tail short; tail-tip round. Forelimb-buds start developing; yolk mass twice as high as body, nearly round. Larva clearly visible through egg jelly and can be moved inside the capsular chamber.	Ground color white-yellowish, with yolk mass more yellow. Dark, irregularly distributed pigmentation on dorsum head and flanks, getting lighter towards the ventrum until total disappearance on ventral side. Eyes white without pigmentation.
28 ($n = 1$)	Head trapezoidal and sloping in profile, snout short and flat, no labial fold visible; gills nearly head high. Dorsal fin higher than head, starting at last third of the trunk; ventral fin shorter than dorsal fin; tail stretching; tail-tip round. Forelimb-buds clearly visible, yolk mass big and round.	Pigmentation similar to stage 27, additionally longitudinal lateral stripe on rib area without pigmentation. Slightly pigmented rim around the eyes forming circle; forelimb-bud base with slight pigmentation on dorsal side.
29 ($n = 2$)	Head more pronounced, labial fold distinct at posterior half of upper jaw; gills developing fimbriae and higher than head; tail getting longer; yolk is less round and oval shaped; forelimb-buds longer with rounded tip; mouth is located on ventral side of head, beneath the snout tip.	Pigmentation getting darker, particularly in the eyes, also slight pigmentation underneath the gular fold.
30 ($n = 2$)	Shape similar to stage 29, forelimb-buds are slightly longer with a dome shaped distal tip; gill rami and fimbriae more developed, dorsal and ventral fin have become larger.	No change in pigmentation.
31 ($n = 2$)	Gills growth; labial folds distinct at posterior half of upper jaw; forelimb cone shaped; tail and fins well developed; dorsal fin starts at middle of the trunk; yolk mass receding.	Pigmentation getting darker, denser pigmentation on dorsum behind head; eyes nearly fully black with white pupil, small, unpigmented stripe from pupil towards ventral side; gill rami slightly pigmented on upper side; fimbriae without pigmentation.
32 ($n = 1$)	Dorsal and ventral fin growing; gular fold clearly visible; mouth orientation is more rostral; two digits developing as small knobs on distal edge of forelimb with a notch forming in the middle.	Pigmentation getting denser on dorsum forming a dark stripe with unpigmented spots; head pigmentation less dense; eyes except for pupil fully pigmented; few dark spots on dorsal and ventral fin and forelimb.
35 ($n = 11$)	Head more depressed, sloping in profile; mouth more pronounced with nares clearly visible; hand with three digits is visible beyond the end of the gills; digits round at the tip; the middle digit the longest; limb with joint, bending at the elbow; yolk mass has almost completely receded; gut getting tubular. Hind limb buds discernible and in some cases elongated, indentation between first two toes in some larvae.	No change in pigmentation.
36 ($n = 3$)	Lateral line organs visible on ventral side of head; mouth open with well-developed teeth; four digits have formed on hand, forelimb turned, palm is facing ventrally. Hind limb with three toes and a knee joint starting to form.	No change in pigmentation.
44 ($n = 4$)	Skin mostly smooth with some warts starting to form; tail long and pointed; limbs well developed with four fingers and five toes; no remains of yolk; head trapezoidal, wide and depressed with a short and flattened snout; dorsal and ventral fin receding; dorsal fin beginning on the first quarter of back and ventral fin beginning above the cloaca; caudal fin higher than head; gills higher than body, with fimbriae still clearly visible.	Pigmentation black and dense over the whole body; lighter on underside of head and ventral side; tip of toes and fingers and labial folds are unpigmented.
45 ($n = 3$)	Skin gets less smooth and more granular and warty; teeth well developed; fins receding; gills atrophy (only stumps left).	Similar to stage 44, but tip of toes and fingers are colored in yellow.

Table 3. Morphological measurements of larvae and respective eggs of *Tylototriton zieglerei* from Ha Giang sorted by stage (mean \pm standard deviation, range in parenthesis, in mm). N: Number of individuals, D: diameter. For abbreviations see Material and Methods. Internostril distance, eye-nostril distance, and snout length were not discernible for stages 27–30 and hind limb length could only be measured from stage 25 onwards.

Stage:	27	28	29	30	31	32	35	36	44	45
(N)	(1)	(1)	(2)	(2)	(2)	(1)	(11)	(3)	(4)	(3)
SVL	7.3	5.30	5.94 \pm 0.21 (5.73–6.15)	5.41	5.95 \pm 0.14 (5.85–6.05)	7.87	7.6 \pm 0.56 (6.32–8.28)	6.89 \pm 0.18 (6.76–7.02)	21.39 \pm 9.37 (10.92–31.94)	34.62 \pm 0.25 (34.44–34.8)
Tal	2.36	1.67	3.71 \pm 0.19 (3.53–3.9)	3.99	3.13 \pm 0.73 (2.61–3.65)	5.59	6.49 \pm 0.85 (5.16–8.7)	5.56 \pm 2.54 (3.76–7.36)	14.04 \pm 7.04 (5.69–23.33)	24.89 \pm 1.37 (23.92–25.86)
TL	9.66	6.97	9.65 \pm 0.52 (9.02–10.05)	9.39	9.08 \pm 0.5 (8.67–9.38)	13.46	14.09 \pm 1.17 (12.17–16.31)	12.45 \pm 3.77 (10.53–15.86)	35.43 \pm 16.42 (17.25–55.11)	59.512 \pm 3.4 (57.82–62.62)
HL	0.73	0.92	0.97 \pm 0.03 (0.94–1)	1.20	1.23 \pm 0.03 (1.21–1.25)	1.95	1.57 \pm 0.26 (1.23–2.14)	1.82 \pm 0.07 (1.77–1.86)	5.15 \pm 1.97 (3.07–7.64)	6.03 \pm 1.15 (5.22–6.84)
HW	0.89	0.95	1.01 \pm 0.18 (0.83–1.19)	1.05	1.36 \pm 0.01 (1.35–1.37)	1.68	1.99 \pm 0.16 (1.78–2.24)	2.22 \pm 0.01 (2.21–2.22)	5.54 \pm 2.37 (2.8–8.36)	8.84 \pm 0.04 (8.81–8.87)
HH	0.66	0.94	0.92 \pm 0.06 (0.86–0.98)	1.16	1.21 \pm 0.01 (1.2–1.22)	1.62	1.5 \pm 0.22 (1.13–1.82)	1.65 \pm 0.1 (1.58–1.72)	2.75 \pm 1.06 (1.37–4.03)	4.65 \pm 0.41 (4.36–4.94)
IoD	0.77	0.75	0.88 \pm 0.12 (0.76–0.99)	1.14	1.09 \pm 0.03 (1.07–1.11)	1.34	1.62 \pm 0.09 (1.46–1.79)	1.81 \pm 0.07 (1.76–1.86)	3.62 \pm 1.62 (1.75–5.55)	5.67 \pm 0.51 (5.31–6.03)
InD	–	–	–	–	0.43	0.57	0.78 \pm 0.18 (0.57–1.28)	0.76 \pm 0.06 (0.72–0.8)	1.69 \pm 0.76 (0.76–2.61)	2.25 \pm 0.48 (1.91–2.59)
EnD	–	–	–	–	0.38	0.44	0.68 \pm 0.08 (0.53–0.78)	0.84 \pm 0.01 (0.83–0.85)	1.63 \pm 0.68 (0.9–2.42)	2.2 \pm 0.56 (1.81–2.6)
SL	–	–	–	–	0.32	0.47	0.78 \pm 0.21 (0.51–1.4)	1.03 \pm 0.08 (0.97–1.09)	2.18 \pm 1.8 (0.44–4.14)	3.21 \pm 0.53 (2.84–3.59)
TH	0.86	1.00	1.11 \pm 0.03 (1.08–1.13)	1.22	1.15 \pm 0.08 (1.09–1.21)	1.85	1.95 \pm 0.32 (1.49–2.55)	2.32 \pm 0.23 (2.16–2.47)	3.46 \pm 1.75 (1.9–5.76)	5.29 \pm 1.32 (4.36–6.23)
TW	0.75	0.69	0.77 \pm 0.04 (0.73–0.8)	0.74	0.65 \pm 0.05 (0.61–0.68)	0.87	1.04 \pm 0.18 (0.81–1.44)	1.1 \pm 0.09 (1.04–1.16)	2.57 \pm 1.32 (1.05–4.08)	3.28 \pm 1.51 (2.21–4.35)
FIL	0.16	0.36	0.43 \pm 0.06 (0.37–0.48)	0.00	0.73 \pm 0.01 (0.73–0.73)	0.82	1.65 \pm 0.26 (1.2–2.24)	2.37 \pm 0.13 (2.28–2.47)	6.84 \pm 2.53 (3.98–9.97)	10.04 \pm 0.35 (9.79–10.29)
HIL	–	–	–	–	–	–	0.66 \pm 0.35 (0.24–1.41)	1.41 \pm 0.17 (1.29–1.54)	6.4 \pm 2.79 (3.69–9.69)	9.63 \pm 0.39 (9.35–9.91)
AD	2.44	2.94	3.22 \pm 0.48 (2.73–3.7)	3.48	3.24 \pm 0.01 (3.23–3.25)	3.91	3.75 \pm 0.43 (3.19–4.75)	3.26 \pm 0.36 (3.01–3.52)	11.74 \pm 5.2 (6.25–17.53)	19.49 \pm 0.31 (19.27–19.71)
egg D	8.8	9.55	10.63 \pm 0.81 (10.05–11.2)	10.57	10.86 \pm 0.14 (10.76–10.96)	10.72	9.78 \pm 0.77 (8.67–11.18)	10.31 \pm 0.65 (9.85–10.77)	–	–

niles of *T. zieglerei* were found in the field, but at stage 45 with total lengths between 57.82–62.62 mm larvae still had not reached metamorphosis.

The detailed descriptions of the available larval stages of *T. zieglerei* are given in Table 2 and respective photographs in Fig. 6. For detailed morphological measurements of the larval stages see Table 3. The overall shape and pigmentation of the larvae remained similar through all stages: head wider than long (with the exception of stages 30 and 32); interocular distance bigger than internostril distance; eye-nostril distance very similar to internostril distance; width of tail base smaller than tail height; tail length smaller than snout-vent length. There was no evidence of balancers throughout the development.

Coloration in life: Body with golden yellowish-brown ground color; venter whitish-transparent. Fingers and toes yellow. Golden spots scattered on dorsal head and trunk, lateral body, tail fin and axilla to throat. Gills yellow with an orange-reddish hue at the edges and on the fimbriae. Ground color turned darker with age. Shortly after metamorphosis the skin was totally black except for yellow fingers, toes, and ventral ridge of tail. At this time the skin started to become less smooth and more granular and warty.

Developmental biology of *T. vietnamensis*

Eggs: The record of one egg directly after deposition had a diameter of 11.97 mm and 0.73 g of weight, while one



Fig. 7. Dark and light phenotypic variations of *Tylostotriton vietnamensis* found at the type locality. Photo M. Bernardes.

egg ready to hatch measured 10.10 mm and weighted 0.56 g. Measurements from random eggs in the field showed an egg diameter ranging between 6.06–13.58 mm (mean 9.73 ± 1.61 mm, $n = 133$) and weight ranging from 0.19–1.15 g (mean 0.48 ± 0.21 g, $n = 133$). Eggs were transparent and clear shortly after egg deposition and later changed to brownish transparent.

Body shape and size of hatched larvae: range of body length at hatching time was 15.59–17.85 mm (mean 17.04 ± 0.85 mm, $n = 5$). Dorsal fin well developed and higher than head, starting at the middle of trunk; ventral fin shorter than dorsal fin; body long and slender; snout short and flat; gills well developed. At stage 33 two fingers were visible in the forelimb and the hind limb bud was already visible. Toes, fingers, and joints were fully developed at stage 41. At stage 44 gills started to atrophy. Efts started to move to land at a size of 44.15 mm with 0.6 g of weight.

Coloration in life: ground color light yellowish ochre; dark pigmentation on dorsal flanks, tail and head; venter slightly transparent to creamy white with no pigmentation; yellow spots scattered on dorsal side of head, body and tail; fingers and toes transparent to yellowish; gills light orange; eyes golden with black pupils. Pigmentation got darker with age turning black shortly before metamorphosis; toes and finger tips remained yellow as well as ventral ridge of tail. However, during field work at the type locality of this species we came across slight phenotypic variations, where larvae were also totally white at older stages (Fig. 7).

Comparison with *T. ziegleri*: The diameter of the gelatinous layer of the egg was bigger in *T. vietnamensis*, as well as sizes of hatchlings. However, the estimated size at metamorphosis is likely bigger in *T. ziegleri*. The

development and body shape of larvae of *T. vietnamensis* were very similar to *T. ziegleri*, with the exception that in *T. vietnamensis* the body is more slender and elongated and the gills more orange than reddish.

Comparisons between the development of *T. ziegleri* and its congeners. *Tylostotriton ziegleri* showed terrestrial oviposition, while *T. taliangensis* and *T. cf. shanjing* showed aquatic oviposition and *T. kweichowensis* and *T. himalayanus* showed both. In *T. podichthys* and *T. panhai* eggs were laid adhered to vegetation, while in *T. ziegleri* eggs were oviposited on the ground. One exceptional clutch of *T. ziegleri* showed eggs in small aggregations, like in *T. podichthys*. *T. ziegleri* had similar clutch sizes compared with *T. hainanensis*, but they were smaller than clutch sizes of *T. kweichowensis* and *T. taliangensis* and bigger than those of *T. vietnamensis*, *T. asperrimus*, *T. wenxianensis*, and *T. himalayanus*. Eggs of *T. ziegleri* were transparent in coloration when young and turned to yellow-brownish when older, like in *T. vietnamensis*, while in *T. himalayanus* eggs were greenish-yellow in color. The comparison between sizes of ova showed larger diameters for *T. ziegleri* in relation to *T. kweichowensis*, *T. asperrimus*, and *T. podichthys*. In relation to the diameter of the gelatinous layer, *T. ziegleri* had similar diameters to those of *T. liuyangensis* and *T. wenxianensis*, which were bigger than those of *T. taliangensis*, *T. kweichowensis*, and *T. podichthys*, and smaller than those of *T. asperrimus*, *T. vietnamensis*, *T. cf. shanjing*, and *T. himalayanus* showed a wider range of egg diameter, both bigger and smaller than those of *T. ziegleri*. Furthermore egg size was related to clutch size, as species with smaller eggs had bigger clutches and vice-versa ($y = -29.68x + 313.64$; $F_{1,5} = 66.85$, $P < 0.001$; $r^2 = 91.7\%$). At hatching time *T. vietnamensis* had the largest larvae, followed in size by larvae of *T. ziegleri*, *T. kweichowensis*, and lastly by *T. himalayanus*. Size at metamorphosis seemed the smallest for *T. shanorum* and *T. vietnamensis*, followed by *T. cf. shanjing*, *T. kweichowensis*, *T. broadoridgus*, and *T. himalayanus*, while in comparison *T. uyenoi* and *T. taliangensis* had the largest sizes at metamorphosis (Table 4).

Generally, the larvae of *T. ziegleri* can be distinguished from the described larvae of the genus *Tylostotriton* by having: 1) a broad head (longer in *T. cf. shanjing*); 2) the interorbital distance wider than internostril distance (similar distances in *T. cf. shanjing*); 3) a pointed tail tip (round in *T. uyenoi*, *T. taliangensis*, *T. cf. shanjing*, and *T. liuyangensis*); 4) the absence of balancers (versus present in *T. uyenoi* and *T. cf. shanjing*); 5) dorsal fin higher than ventral fin (almost identical height in *T. liuyangensis*); 6) tail shorter than SVL (tail longer than SVL in *T. himalayanus*); 7) reddish gills (versus orange in *T. vietnamensis*); 8) advanced larval stages with dark ground color with the exception of yellow digits and ventral fin (versus orange digits and fin in *T. broadoridgus*; yellow at head,

Table 4. Reproduction data of *Tylototriton* representatives (after Bourret 1942; Khatiwada et al. 2015; Kuzmin et al. 1994; Mudrack 2005; Nishikawa et al. 2013a, 2014, 2015; Phimmachak et al. 2015; Shen et al. 2012; Sparreboom 2014; Yang et al. 2014; Zhao 1988; Ziegler et al. 2008; and after own data for *T. vietnamensis* in comparison with *T. ziegleri*). * – based on the description of a single larva; ** – based on the description of two juveniles. All measurements in mm. For abbreviations see Material and Methods.

	Clutch size (Egg nrs.)	Oviposition site	Jelly layer diameter	Ovum diam- eter	TL hatchling	Body shape and size of larvae	Larvae coloration	Differences to <i>T. ziegleri</i>
Subgenus								
<i>Yaotriton</i>:								
<i>T. asperrimus</i>	30–52	–	10	3.0–3.4	–	–	–	bigger diameter of ge- latinous layer; ovae di- ameter and clutch size smaller
<i>T. broador- idgus</i>	–	–	–	–	–	gills start to at- rophy at 62 mm TL	orange coloration on digits and ven- tral fin	yellow coloration on digits and ventral fin in <i>T. ziegleri</i>
<i>T. hainanensis</i>	58–90	–	–	–	–	–	–	similar clutch sizes
<i>T. kweichow- ensis</i>	121–141	in water, on moist soil or under large stones nearby water	6.2–6.5	2.3–3.4	12	~ 62 mm TL at metamorphosis	–	bigger clutch size; di- ameter of gelatinous layer, ovae and hatch- ling size smaller, in <i>T.</i> <i>ziegleri</i> only terrestrial oviposition
<i>T. liuyangensis</i>	–	on land	7.8–8.1	–	–	–	dorsal and ventral fins almost identi- cal in height; tail tip rounded	dorsal fin higher and tail tip pointed in <i>T.</i> <i>ziegleri</i>
<i>T. panhai</i>	–	adhered to sticks slightly above the water surface	–	–	–	–	–	egg deposition terres- trial, mostly on rock substrate in <i>T. ziegleri</i>
<i>T. podichthys</i>	–	adhered to vegetation (individu- ally or in groups of up to three)	5.0 ± 0.3	2.9 ± 0.2	–	–	–	eggs not adhered to vegetation and egg ag- gregations only in one exceptional case in <i>T.</i> <i>ziegleri</i> ; gelatinous layer and ovum diam- eter larger
<i>T. vietnamensis</i>	5–85	on land	6.06–13.58	–	15.59–17.85	44.15 mm at metamorpho- sis; slender and elongated body	orange colored gills	smaller clutch sizes; diameter of gelatinous layer and hatchling sizes bigger; body more slender and elon- gated; estimated TL at metamorphosis for <i>T.</i> <i>ziegleri</i> bigger; gills more reddish than or- ange in <i>T. ziegleri</i> .
<i>T. wenxianensis</i>	56–81	on land or in transition to water	7–8	3	–	–	–	clutch size slightly smaller

parotids, vertebral ridge, rib nodules, limbs and tail in *T. uyenoi* and *T. shanorum*; brighter coloration laterally in the rib area in *T. cf. shanjing*); and 9) being less slender than larvae of *T. vietnamensis*.

Discussion

Tylototriton ziegleri occurred at elevations congruent with the data provided by Nishikawa et al. (2013b). Occurrences at higher elevations were also found in Cao Bang Province, but not as high as the 1,600 m above sea level reported by Sparreboom (2011) at Mt. Pia Oac in Nguyen Binh district, Cao Bang Province. *Tylototriton vietnamensis* was always found at lower elevations, however, the population from Lang Son Province at 980 m

above sea level was found higher than previous records for this species, setting a new elevational record.

The breeding season of *T. ziegleri* in northern Vietnam was previously thought to last from April to May (Nishikawa et al. 2013b); based on our new findings this period lasts longer, from April to July. Likewise, the breeding season of *T. vietnamensis* was recorded to last from June to July (Böhme et al. 2010), but our records show that it starts already in April.

We present for *T. ziegleri* a broader range for the distance of clutches to water with 10–100 cm instead of the 50–60 cm reported by Nishikawa et al. (2013b). The average amount of eggs in a clutch unable to produce viable offspring is still unknown, but might strongly increase by mycosis infection, as observed in one clutch

Table 4 (continued). Reproduction data of *Tylototriton* representatives (after Bourret 1942; Khatiwada et al. 2015; Kuzmin et al. 1994; Mudrack 2005; Nishikawa et al. 2013a, 2014, 2015; Phimmachak et al. 2015; Shen et al. 2012; Sparreboom 2014; Yang et al. 2014; Zhao 1988; Ziegler et al. 2008; and after own data for *T. vietnamensis* in comparison with *T. ziegleri*). * – based on the description of a single larva; ** – based on the description of two juveniles. All measurements in mm. For abbreviations see Material and Methods.

Subgenus									
<i>Tylototriton</i> :									
<i>T. anguliceps</i> *									
—	—	—	—	—	—	dorsal fin higher than ventral fin; tail tip pointed	fingers and toes yellow	very similar body shape and coloration of larvae	
<i>T. himalayanus</i>									
26–60	in water or on land	6–10 (greenish-yellow in color)	—	—	10.9 ± 0.62	TaL ≤ SVL; 62.5 ± 2.67 mm at metamorphosis	—	clutch size smaller; wider range of egg diameter; smaller hatchlings; in <i>T. ziegleri</i> only terrestrial oviposition, eggs transparent and later brownish, and SVL > TaL	
<i>T. cf. shanjing</i>									
—	in water (e.g., on submerged vegetation)	6–10	—	—	—	HL > HW; IoD > EnD; InD ~ IoD; TaL ≤ SVL; rounded tail tip; presence of balancers; ~ 52 mm at metamorphosis	lack of dark pigmentation in the rib area, which in older larvae might generate the colorful flank tubercles	wider range of egg diameter; larvae with presence of balancers and lighter ground color; in <i>T. ziegleri</i> terrestrial oviposition, HW > HL and tail tip pointed	
<i>T. shanorum</i> **									
—	—	—	—	—	—	34 and 43 mm (juveniles)	bright yellow coloration on dorsal head, lips, parotids, vertebral ridges, rib nodules, limbs, vent region and whole tail	estimated TL at metamorphosis bigger for <i>T. ziegleri</i> ; juveniles of <i>T. ziegleri</i> completely black except for yellow coloration on fingers and tail fin	
<i>T. taliangensis</i>									
250–280	in water (individually on water plants)	2–2.2	—	—	—	dorsal and ventral fins almost in parallel; tail tip rounded; larvae overwinter and metamorphose the following year with 59–72 mm sizes	—	oviposition terrestrial in <i>T. ziegleri</i> ; egg diameter smaller; clutch size bigger; tail tip pointed and dorsal and ventral fins not parallel in <i>T. ziegleri</i>	
<i>T. uyenoii</i> *									
—	—	—	—	—	—	presence of balancers in early stages; tail tip round	anterior head, parotids, vertebral ridge, rib nodules, limbs and tail yellow in advanced larval stages	absence of balancers, tail tip pointed and yellow coloration only on fingers and tail fin in <i>T. ziegleri</i>	

in Bao Lac district.

Aquatic breeding sites with alkaline pH values and higher carbonate hardness (Quang Ninh, Cao Bang and Ha Giang provinces) were associated with the geological substrate of the areas, mainly limestone rock (Sterling et al. 2006). These karst limestone areas are characterized by thin layers of surface soils, periods of severe soil dryness due to quick drainage of water and erosion of subsurface rock material, resulting in extensive cave systems with underground streams. Firstly, this explains the significantly deeper ponds found in habitats of *T. ziegleri* and secondly the deficiency of soil, leaving the parent rock exposed and explaining the choice of substrate for oviposition. *Tylototriton vietnamensis* on the other hand occurs on soils with granite parent rock material (Bernardes et al. 2017) which are more acidic and have low

cation exchange capacity (Ulrich 1991). Ponds inhabited by *T. ziegleri* had higher nitrite and nitrate concentrations than ponds inhabited by *T. vietnamensis*. High levels of these nutrients have usually an anthropogenic origin, like leaching of nitrogen from manure and mineral fertilizers from upstream villages or agricultural fields. High concentrations of nitrate and nitrite can have negative effects on aquatic amphibian larvae, although there are interspecific differences in species’ sensitivity (Marco et al. 1999), which at this point do not seem to negatively affect the investigated species.

The additional finger found in one individual at stage 44 can have numerous causes, as malformations in amphibians have not yet been fully investigated (Blaustein and Johnson 2003). Polydactyly was, for exam-

ple, associated with ultraviolet radiation (Ankley et al. 2000, 2002), chemical contamination (Burkhart et al. 1998; Gardiner and Hoppe 1999), and parasitic infection (Johnson et al. 1999; Sessions and Ruth 1990). However, developmental abnormalities found in amphibian populations have typical frequencies of 0–3% and are only considered abnormally high when exceeding 5% (Piha et al. 2006). Although field observations revealed that this type of malformation is quite common among adults of *Tylototriton* in Vietnam (M. Bernardes, pers. obs.), more studies have to be conducted to conclude whether these abnormalities are above natural levels.

The diagnostic characteristics of the different developmental stages in *T. ziegleri* corresponded well to the staging system adopted by Grosse (1997, 2013). Noticeable differences concern the earlier development of hind limbs (at stage 36 hind limbs had already developed three toes, while according to Grosse the hand development is finished before hind limb buds occur) and the absence of balancers. Balancers are usually present in salamander larvae that develop in lentic habitats sustaining the hypotheses that these structures are adaptive to still waters and non-functional in flowing waters (Crawford and Wake 1998). This theory does not seem to be verified in *T. vietnamensis*, a species that breeds in lentic habitats, but could explain the absence of balancers in *T. ziegleri*, a species able to reproduce both in lentic as in lotic habitats.

The body length of hatchlings of *T. ziegleri* was on average 30% smaller than the 21–22 mm reported by Sparreboom et al. (2011). According to these authors the larvae completed their metamorphosis at sizes of 43–62 mm. In our collection the biggest larva had not completed metamorphosis at stage 45 with 60 mm total length. However, length at metamorphosis seems to be a variable feature in *Tylototriton* representatives and apparently also depends on parameters such as feeding regime, temperature, etc. Total lengths in eight metamorphlings of *T. vietnamensis* reared in captivity by F. Pasmans varied between 49.9 and 65.8 mm. Further, observations in captive reared *T. wenxianensis* larvae revealed large variation in the stages at which the larvae hatched and consequently also in the total length at hatching (F. Pasmans, pers. obs.).

The morphological similarity within the *T. asperrimus* group in terms of body shape and coloration of adults makes it especially difficult to tell species apart (Stuart et al. 2010). Morphological similarity is even higher in larvae as this study shows. The larval development of *T. ziegleri* is still not completely recorded, as several stages still are unknown. Nevertheless our data allow clear morphological comparisons of developmental stages within this genus. Egg capsule diameter seems to be larger in species with terrestrial oviposition compared to species with aquatic oviposition. We therefore hypothesize that eggs of species with terrestrial oviposition are characterized by an extra thick gelatinous layer to prevent exsic-

cation of the developing larva, and therefore this trait might be a good indicator for the species' behavior. *Tylototriton ziegleri* had the biggest clutches of all land-laying species and amongst the sub-genus *Yaotriton*, while clutches of *T. vietnamensis* are among the smallest. The wide ranges in clutch sizes seen in *T. vietnamensis* and *T. cf. shanjing* might be related to the big sample size in the first case and associated with hidden cryptic diversity in the second. These comparisons must be regarded with caution, since accumulating evidence suggests that the description of the larval development of *T. cf. shanjing* by Ziegler et al. (2008) was based on a species complex (e.g., Nishikawa et al. 2013a). More information on the larval development in the genus *Tylototriton* is certainly needed.

Conclusion

Tylototriton ziegleri is a small-ranged species known only from four localities in the North of Vietnam, none of them within protected areas. The species is not yet listed in the IUCN Red List, although it is likely under threat of extinction. Like *T. vietnamensis* it is in demand for the international pet trade as well as the Chinese market (Rowley et al. 2016). Despite morphological conservatism in particular within larval stages, our data clearly confirm contrasting habitat requirements between these cryptic species, both in adults and larval stages. Therefore, our results provide useful guidance to establish proper captive conditions for these two species with strongly deviating breeding requirements. This is in particular important as *Tylototriton* is known for its cryptic diversity, as it can be seen for example with what was in the past thought to be the single species *T. shanjing*. From this morphological cryptic group several species have been described, like *T. panhai* and *T. uyenoii* (Nishikawa, Khonsue, Pomchote, and Matsui, 2013), *T. anguliceps* (Le, Nguyen, Nishikawa, Nguyen, Pham, Matsui, Bernardes, and Nguyen, 2015), and *T. podichthys* (Phimmachak, Aowphol, and Stuart, 2015), while *T. v. pulcherrima* was considered to be conspecific (Nishikawa et al. 2013a). Meanwhile the *T. shanjing* complex is widely distributed in zoological gardens, but origin and specific identification is in most cases uncertain, as well as information about potential captive hybridization. It is virtually impossible to identify representatives of the *T. shanjing* group without a comprehensive genetic screening. This negative impact on proper conservation breeding measures is yet aggravated by the lack of information regarding origin, natural history data, and data on different habitat adaptations in the field.

Our study describes the different ecological adaptations to strongly contrasting environmental conditions of two morphologically similar species. We highlight the necessity to improve the knowledge on the natural history of the *Tylototriton* species, not only for enhanced *ex situ* measures (viz. husbandry and conservation breeding,

see Ziegler et al. 2016), but also for *in situ* approaches, such as supporting the establishment of new reserves, or extending the area of existing ones, as the populations of *T. ziegleri* from Quan Ba and Bac Quang districts occur in the vicinity of protected areas (Bat Dai Son Nature Reserve in Ha Giang Province and Cham Chu Nature Reserve in Tuyen Quang Province, respectively) but are not included in one.

Acknowledgements.—We are grateful to the directorates and staff of Tay Yen Tu, Yen Tu, Dong Son – Ky Thuong and Bat Dai Son nature reserves, Forest Protection Departments of Bac Giang, Quang Ninh, Lang Son, and Ha Giang provinces for support of our field work and issuing the required permits. We cordially thank S.V. Nguyen, C.X. Le (IEBR, Hanoi), T. Pagel and C. Landsberg (Cologne Zoo) for support of our research. We thank M. van Schingen and H.T. An for their assistance in the field and H.T. Ngo for laboratory assistance. This research was partially funded by the Cologne Zoo (Germany), the European Association of Zoos and Aquaria (EAZA), the Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT), the Amphibian Conservation Fund of German Zoo Associations, private participants in the German-speaking region as well as Stiftung Artenschutz, and the University of Cologne.

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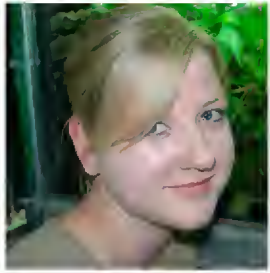
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Larval development and breeding ecology of Ziegler's Crocodile Newt



Marta Bernardes is a Ph.D. candidate at the Zoological Institute of the University of Cologne and the Cologne Zoo, Germany. She has a M.S. degree in Conservation Biology from Lisbon University, Portugal. Since 2007 she has been engaged in the research of amphibians and reptiles and their natural environment with a main interest in ecology and conservation. In 2010 she joined the working group of Thomas Ziegler and initiated ecological research projects in Southeast Asia with a main focus on the Salamandridae family from Vietnam.



Anna Rauhaus started her career at the Aquarium/Terrarium Department of the Cologne Zoo in May 2011 and is head keeper of the Terrarium section since 2014. Her focus of expertise is keeping and breeding of amphibians, monitor lizards, snakes, and crocodilians as well as behavioral training. She also trains keepers and helps to build amphibian and reptile facilities within the framework of Cologne Zoo's conservation projects in Vietnam. Since 2011 she has been involved in 35 herpetological publications with a focus on zoo biology, with ten of them dealing with captive breeding, larval development, and diversity of tropical species, in particular Vietnamese amphibians.



Clara Michel performed her bachelor thesis "Larval development and ecological niche of Ziegler's Crocodile Newt (*Tylototriton ziegleri*)," which was submitted in February 2015 at the University of Cologne, under the supervision of Profs. Thomas Ziegler and Michael Bonkowski, as well as co-supervised by Marta Bernardes and Anna Rauhaus.



Cuong The Pham is a Ph.D. candidate and researcher of the Institute of Ecology and Biological Resources (IEBR) - Vietnam Academy of Science and Technology (VAST). He is member of the Cologne Zoo's Biodiversity and Nature conservation projects in Vietnam. Cuong has published several papers, mainly dealing with Vietnam's herpetodiversity. Cuong is very experienced in biodiversity and field research and conducted numerous field surveys in Vietnam.



Truong Quang Nguyen is a researcher at the Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology (VAST) and is a member of the Biodiversity and Nature Conservation projects of the Cologne Zoo. He finished his Ph.D. in 2011 at the Zoological Research Museum Alexander Koenig (ZFMK) and the University of Bonn, Germany (DAAD Fellow). From 2011 to 2014 he worked as a postdoctoral student in the Zoological Institute at the University of Cologne. Truong has conducted numerous field surveys and is the co-author of seven books and more than 150 papers relevant to the biodiversity research and conservation in Southeast Asia. His research interests are systematics, ecology, and phylogeny of reptiles and amphibians from Southeast Asia.



Minh Duc Le has been working on conservation-related issues in Southeast Asia for more than 15 years. His work focuses on biotic surveys, wildlife trade, and conservation genetics of various wildlife groups in Indochina. He is currently working on projects which characterize genetic diversity of highly threatened reptiles and mammals in the region. Minh has pioneered the application of molecular tools in surveying critically endangered species in Vietnam. Minh has long been involved in studying the impact of the wildlife trade on biodiversity conservation in Vietnam, and is developing a multidisciplinary framework to address the issue in the country.



Frank Pasmans is a veterinarian and director of the laboratory of veterinary bacteriology and mycology at Ghent University (Belgium). He has had a lifelong obsession for amphibians, notably urodeles. His research currently focuses on fungal infections in amphibians. By studying fundamental processes of host – pathogen – environment interactions, this research aims at developing long-term sustainable measures to mitigate the impact of fungal diseases on amphibian communities.



Michael Bonkowski is Professor for Terrestrial Ecology in the Institute of Zoology at the University of Cologne. His research spans from soil biodiversity and function to sustainable soil management, and more recently included studies on the biodiversity and function of tropical ecosystems in Southeast Asia. One aim is to better understand the mechanisms of community assembly of amphibians and reptiles in tropical rain forests of Vietnam and Laos. This research is focusing on patterns of geographic genetic differentiation and attributes of the ecology and life history of amphibians and reptiles. This work is placed in the context of ecosystem-level consequences of biodiversity loss due to factors such as habitat degradation and destruction, and on shifts in tolerances to changing temperatures as expected by global change.



Thomas Ziegler has been the Curator of the Aquarium/Terrarium Department of the Cologne Zoo since 2003 and is the coordinator of the Cologne Zoo's Biodiversity and Nature Conservation Projects in Vietnam and Laos. Thomas studied biology at the University Bonn (Germany), and conducted his diploma and doctoral thesis at the Zoological Research Museum Alexander Koenig in Bonn, with focus on zoological systematics and amphibian and reptile diversity. He has been engaged with herpetodiversity research and conservation in Vietnam since 1997. As a zoo curator and project coordinator he tries to combine *in situ* and *ex situ* approaches, viz., to link zoo biological aspects with diversity research and conservation, both in the Cologne Zoo, in rescue stations and breeding facilities in Vietnam and in Indochina's last remaining forests. He is Professor at the Zoological Institute of Cologne University. Since 1994, Thomas has published 370 papers and books, mainly dealing with herpetodiversity.



On the distribution of the Himalayan Stripe-necked Snake *Liopeltis rappi* (Günther, 1860) (Serpentes: Colubridae) in Nepal

¹Santosh Bhattarai, ²Kul Bahadur Thapa, ¹Lina Chalise, ¹Aashish Gurung, ¹Chiranjibi Prasad Pokheral, ³Naresh Subedi, ²Tej Bahadur Thapa, and ⁴Karan Bahadur Shah

¹National Trust for Nature Conservation-Biodiversity Conservation Center (NTNC-BCC), Ratnanagar-18, Sauraha, Chitwan-44204, NEPAL

²Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, NEPAL ³National Trust for Nature Conservation-Khumaltar, Lalitpur, NEPAL ⁴Natural History Museum, Tribhuvan University, Swoyambhu, Kathmandu, NEPAL

Abstract.—The distribution of the Himalayan Stripe-necked Snake (*Liopeltis rappi*) is poorly documented. We summarize the distribution of this little known snake in Nepal and provide a new locality record from Kabilas, Chitwan, Nepal. Compiled observations presented here suggest that the species is more widely distributed and we call for additional surveys and a systematic inventory.

Keywords. Chitwan-Annapurna landscape, conservation, reptile, biodiversity, South Asia, Squamata

Citation: Bhattarai S, Thapa KB, Chalise L, Gurung A, Pokheral CP, Subedi N, Thapa TB, and Shah KB. 2017. On the distribution of the Himalayan Stripe-necked Snake *Liopeltis rappi* (Günther, 1860) (Serpentes: Colubridae) in Nepal. *Amphibian & Reptile Conservation* 11(1) [General Section]: 88–92 (e139).

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Received: 24 January 2017; **Accepted:** 15 May 2017; **Published:** 30 May 2017

Introduction

The Himalayan Stripe-necked Snake *Liopeltis rappi* (Günther, 1860) is a small, slender, non venomous snake distributed through the Himalayas of Nepal and India (Smith 1943; Wallach et al. 2014). Günther (1860) described this species from Sikkim, India and originally it was described as *Ablabes rappi*. Later, Wall (1921) placed it in the genus *Liopeltis*. Reporting of its distribution has been sporadic both in time and space and it is an uncommonly encountered species. Previously it has been reported in India from Sikkim (Chhetri et al. 2011; Günther 1860), Darjeeling (Wall 1909), and Himanchal Pradesh (Saikia et al. 2007; Smith 1943) in India and Chitwan (Schleich and Kästle 2002; Shrestha 2001), Khotang, Terhathum, Shankhuwasabha (Rai 2003), Kaski (Shah and Tiwari 2004), and Palpa (Thapa 2016) in Nepal. The information on diversity and species richness of the ophidian fauna in Nepal is scanty. Nepalese snakes are represented by Palearctic, Ethiopian, and Oriental species (Schleich and Kästle 2002). Recently, Kästle et al. (2013) listed the occurrence of 82 species in Nepal, and questioned the occurrence of 14 snake species included in the list of Schleich and Kästle (2002) and Shah and Tiwari (2004) due to several taxonomic revisions. Most of the herpetological expeditions have

focused in eastern and central Nepal. These expeditions usually report new taxa or new distribution records for the country. For example, Sharma et al. (2013) and Pandey (2015) recently added two new snake species record viz *Bungarus sindamus walli* (Boulenger 1897) and *Oligodon cylcurus* (Cantor 1839) respectively for Nepal. We here add one more significant record of *Liopeltis rappi* from Kabilas, Chitwan which is a part of the Chitwan-Annapurna Landscape.

The Chitwan-Annapurna Landscape (CHAL) is located in central Nepal and it includes all or part of 19 districts covering an area of 32,057 km², with elevations ranging from 200 m to 8,091 m asl. The landscape is drained by eight major perennial rivers and their tributaries from the broader Gandaki River system. The CHAL experiences a range of climates from subtropical in the lowlands to alpine in the high mountains, and cold and dry in the trans-Himalayan region. It is suggested that climate change is a major cause behind changes in floral and faunal diversity in the CHAL (MoFSC 2015). In this regard, twelve permanent plots have been established in the CHAL to study the vulnerability of species due to climate change or other factors. These plots lie in Barandabhar, Kaule, and Kabilas of Chitwan district, Tilakpur and Asardi of Palpa district, Panchase of Syangja district and in Mustang district.



Fig. 1. *Liopeltis rappa* from Dhodeni, Kabilas, Chitwan. Photo Santosh Bhattarai.

Materials and Methods

We examined a recently dead specimen of *Liopeltis rappa* found at Kabilas-09, study site at Dhodeni, Chitwan (GPS: 27.78418°N 84.51605°E, elevation, 978 m, WGS: 1984) during survey work monitoring climate effects on one of the permanent plots. It was found dead on a trail in an abandoned cultivated field at 12:19 h on 16, July 2016. The cause of death was not readily apparent.

We recorded morphometric and meristic data for this specimen: dorsal scale rows at three points, approximately one head length posterior to the head, midbody, and one head length anterior to the vent. Ventral scales were counted following Dowling (1951), subcaudals and dorsal scales. Snout Vent Length (SVL) and Total Length (TTL) were measured with a thread, later scaling it to a

metallic ruler. Digital camera Canon-65 X optical zoom was used for photographic record, description of colors, and patterns. The broad habitat type and plant species were recorded at the place where the specimen was located.

Results

The small snake measured 462 mm SVL, 572 mm TTL and was identified as *Liopeltis rappa* (Fig. 1) based on the following combination of characteristics: head short and not distinct from the neck, round pupil, nostrils large and between two nasals, dorsal scales 15:15:15, all smooth; ventrals 176; subcaudals 60; all paired. There were six supralabials, with the 3rd-4th contacting the eye, 5th largest, a single preocular, and two post oculars. The dorsal

Table 1. Locality records of *Liopeltis rappa* in Nepal.

S.N.	Locality	Habitat	Coordinates	Elevation	District	Source
1	Lasune	Small town	27.14586°N, 87.46302°E	2,250 m	Tehrathum	Rai 2003
2	Chisapani /Nagdhunga	Paddy field	26.96709°N, 86.88333°E	1,600 m	Khotang	Rai 2003
3	Makalu Barun NP	—	27.66266°N, 87.10458°E	2,200 m	Sankhuwasabha	Rai 2003
4	Ghandruk	—	28.46638°N, 83.71421°E	2,972 m	Kaski	Shah and Tiwari 2004
5	Khaliban	Waste land	27.85624°N, 83.84418°E	813 m	Palpa	Thapa 2016
6	Chappani, Jhirbhanjyang	Waste land	27.89579°N, 83.56964°E	1,056 m	Palpa	Thapa 2016
7	Bandipokhara, Lipindevi	Waste land	27.86725°N, 83.50192°E	1,498 m	Palpa	Thapa 2016
8	Tansen, Bhusaldanda	Small town	27.87159°N, 83.55696°E	1,292 m	Palpa	Thapa 2016
9	Khanigaun, Khiluadada	Waste land	27.91685°N, 83.55087°E	1,139 m	Palpa	Thapa 2016
10	Dhodeni/Kabilas	Cultivated land	27.78418°N, 84.51605°E	978 m	Chitwan	This study

Distribution of *Liopeltis rappi* in Nepal

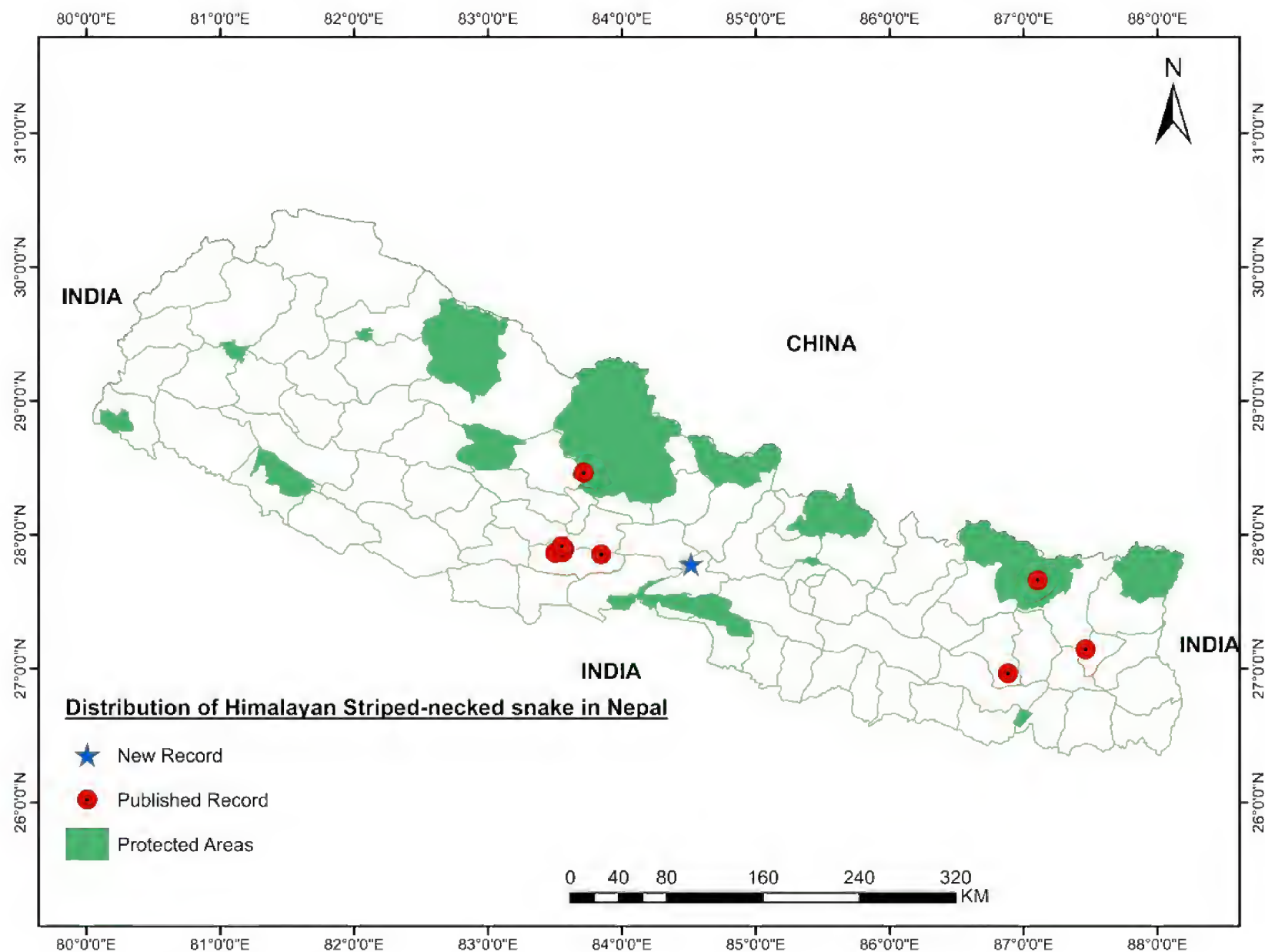


Fig. 2. Distribution of *Liopeltis rappi* in Nepal.

color was uniform coffee brown and the venter was yellow. The specimen represents the 10th locality record of *L. rappi* for Nepal. Figure 2 provides an overview of distribution of *L. rappi* in Nepal and Table 1 summarizes the locality records.

Habitat and herpetological community: The specimen was recorded in a seasonally abandoned cultivated land (Fig. 3). The broad habitat type of the locality was broadleaf mixed forest. The plant species recorded at the site were *Lantana camara*, *Eupatorium* sp., and *Ageratum* sp. Other herpetofauna recorded at the same locality comprised of anurans: *Duttaphrynus melanostictus* (Schneider 1799), *Microhyla ornata* (Duméril and Bibron 1841), *Sphaerotheca maskeyi* (Schleich and Anders 1998), *S. breviceps* (Schneider 1799), *Polypedates maculatus* (Gray 1830), *Amolops marmoratus* (Blyth 1855), and *Fejervarya* sp. (Bolkay 1915); lizards: *Calotes versicolor* (Daudin 1802), *Eutropis carinata* (Schneider 1801), *E. macularia* (Blyth 1853), and *Laudakia tuberculata* (Gray 1827); and snakes: *Amphiesma stolatum* (Linnaeus 1758), *Dendrelaphis tristis* (Daudin, 1803), and *Sibynophis collaris* (Gray 1853).

Discussion

Distribution: The closest published records to the CHAL for *Liopeltis rappi* are Ghandruk, Kaski 100 NW (Shah and Tiwari 2004) and Palpa 95 km W (Thapa 2016). Ear-

lier published records of *L. rappi* (Günther 1860; Anderson 1871; Boulenger 1890; Smith 1943; Krammer 1977; Shrestha 2001) did not mention data on locality records for Nepal. Shah (1995) and Schleich and Kästle (2002) reported the occurrence of *L. rappi* in Sauraha, Chitwan. Later, Shah, and Tiwari (2004) verified the record from Chitwan as a wrongly identified specimen and mentioned the new occurrence locality of this species from Ghandruk, Kaski. Captain (2010) also questioned the occurrence of *L. rappi* in Sauraha, Chitwan as this species is thought to be distributed at higher elevations. We agree with Shah and Tiwari (2004) and Captain (2010), hence, remove the occurrence of *L. rappi* from Sauraha and report our observation locality, Kabilas-09, Dhodeni as the first confirmed record from Chitwan. Our observation locality is 978 m asl and ranges within the elevation record of Thapa (2016). The elevational records range from 813 m to 2,972 m, demonstrating that the species probably exhibits a wider distribution in Nepal.

Conservation status: The IUCN (2016) has assessed *L. rappi* as a Data Deficient (DD) species and its population trend unknown. Of ten distribution localities, two localities Ghandruk and Makalu Barun National Park are within protected areas. Other localities are designated as either cultivated land, waste land, or small towns, and these sites were identified as important for the conservation of the species in Nepal, as the cultivated lands are being mechanized and villages are growing larger and



Fig. 3. Habitat of *Liopeltis rappa* at Dhodeni, Kabilas, Chitwan. Photo Santosh Bhattarai.

into towns. The present record of the dead specimen from cultivated land tends to show the possible ignorance of local people in the survival of the species. Thapa (2016) recorded five specimens from Palpa, of which four were found killed by local people and a single live specimen from Khaliban. People in this area kill snakes at the moment they encounter them as standard practice in the culture. This rampant killing of snakes, including *L. rappa* by local people, is an observed threat in the CHAL. All snakes are believed by the local people to be venomous despite the fact that only 17 species of snakes in Nepal are venomous (Sharma et al. 2013). Outreach activities among farmers, local communities, in schools, and colleges should focus on the good ecosystem function of snakes and basic identification tools of snakes would be instrumental in better protecting the snake fauna of the CHAL. Our finding indicates that a countrywide detailed herpetological survey would be beneficial to better understand the ecology, distribution pattern, threats, and conservation status of *L. rappa* in Nepal.

Acknowledgements.—This is an offshoot of the Project “Climate Change Monitoring on Permanent Plots in Chitwan-Annapurna Landscape” funded by USAID-Hariyo Ban Program/NTNC. We thank Harkaman, Kapil, Pratigya, Biraj, Trishna, Pramod, Deepu, Tika, Om, Binod, and Ramesh for field support. NTNC-BCC provided logistic support and field coordination. We acknowledge Mark O’Shea and George Zug for comments on earlier drafts and Frank Tillack for related literature and discussion which benefitted the manuscript. We thank Pabitra Gotame for her help in the field and map preparation.

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Santosh Bhattarai is currently working as a Conservation Officer at the National Trust for Nature Conservation- Biodiversity Conservation Center (NTNC-BCC), Sauraha, Chitwan, Nepal. He is particularly interested in understanding evolutionary and ecological drivers of amphibians and reptiles for which species diversify and evolve through time and space.



KB Thapa is a graduate in zoology from Tribhuvan University of Nepal. He is impassioned about wildlife research, conservation, and particularly herpetology. His study on amphibians and reptiles in his M.Sc. thesis was guided by this same passion. In that study he reported a total of forty eight species of herpetofauna of which sixteen species had national and/or global altitudinal/latitudinal and/or longitudinal distribution range extension. At present, he is engaged with one of the leading national non-government organizations working in the field of nature conservation and research.



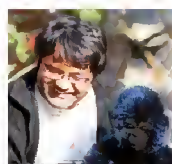
Lina Chalise holds a M.Sc. in Biodiversity and Eenvironmental Mmanagement. She is currently working as a conservation officer at the National Trust for Nature Conservation- Biodiversity Conservation Center, Sauraha, Chitwan, Nepal.



Aashish Gurung graduated in Environmental Science and Natural Resource Management from Kathmandu University. Currently, he works as a Conservation Officer at the National Trust for Nature Conservation– Biodiversity Conservation Center, Sauraha, Chitwan, Nepal.



Chiranjibi Prasad Pokheral currently works as a Project Manager at the National Trust for Nature Conservation–Biodiversity Conservation Center, Sauraha, Chitwan, Nepal. He completed his Ph.D. in 2012 and has more than two decades of experience in species conservation and management in Nepal. He is focused on managing biodiversity projects in Chitwan-Parsa complex.



Naresh Subedi completed his Ph.D. in 2012 and is currently based at the NTNC-central office, Kathmandu and works as a Conservation Program Manager. His earlier research was focused on the impact of invasive species on native wild animals and subsequent conservation measures.



Dr. T.B. Thapa is a professor of wildlife at Central Department of Zoology, Tribhuvan University in Kathmandu, Nepal. He has made significant contribution in wildlife research and in the formulation of national conservation strategies of many species. He has also been instrumental in guiding many new researchers fascinated in wildlife.



Karan Bahadur Shah is a Professor of Zoology at Tribhuvan University, Natural History Museum, Swoyambhu, Kathmandu. He has described several reptiles in Nepal and authored a book “*Herpetofauna of Nepal: Conservation Companion*” published by IUCN Nepal.



The herpetofauna of central Uzbekistan

^{1,2,*}Thomas Edward Martin, ^{1,2}Mathieu Guillemin, ^{1,2}Valentin Nivet-Mazerolles, ^{1,2}Cecile Landsmann, ^{1,2}Jerome Dubos, ^{1,2}Rémy Eudeline, and ³James T. Stroud

¹Emirates Centre for the Conservation of the Houbara, Urtachol massif, Karmana Shirkat farm, Navoi Region, REPUBLIC OF UZBEKISTAN
²Reneco for Wildlife Preservation, PO Box 61 741, Abu Dhabi, UAE. ³Department of Biological Sciences, Florida International University, Miami, Florida, USA

Abstract.—The diverse habitats of central Uzbekistan support a rich herpetofaunal community, but distributions and relative abundances of the species comprising this community remain poorly known. Here, we present an annotated species inventory of this under-explored area, with detailed notes on distributions and population statuses. Fieldwork was concentrated in southern Navoi and western Samarkand provinces, although some records were also made in the far north of Navoi province, near the city of Uchkuduk. Data were collected between March and May/June in 2011, 2012, and 2013, with herpetofaunal records being made opportunistically throughout this period. Survey effort was concentrated in semi-desert steppe habitats, especially the Karnabchul steppe area located to the south of the city of Navoi and an expanse of unnamed steppe located to the north of Navoi. Further records were made in a range of other habitat types, notably wetlands, sand dune fields, and low rocky mountains. Total fieldwork equated to approximately 8,680 person-hours of opportunistic survey effort. In total, we detected two amphibian and 26 reptile species in our study area, including one species classified as Globally Vulnerable by the IUCN. We present distributional data supporting the first record of regional range extensions of five species from within our study area. Our results represent the most detailed data concerning reptile and amphibian diversity and distributions produced from Uzbekistan in recent years. We conclude by recommending that further, systemized survey work needs to be conducted within the area to supplement our findings with more robust estimates of species abundances supported by more detailed information on species-habitat relationships.

Keywords. Central Asia, faunistics, inventory, steppe, distribution, survey

Citation: Martin T, Guillemin M, Nivet-Mazerolles V, Landsmann C, Dubos J, Eudeline R, and Stroud J. 2017. The herpetofauna of central Uzbekistan. *Amphibian & Reptile Conservation* 11(1) [General Section]: 93–107 (e140).

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Received: 08 April 2016; **Accepted:** 01 Aug 2016; **Published:** 14 June 2017

Introduction

Central Asia (defined in this study as the five Central Asian Republics and Afghanistan) encompasses a wide range of habitats, which in turn support a rich and, in some areas, highly endemic biodiversity. The region's ecological importance is reflected by it encompassing five “global 200” terrestrial ecosystems (Olson and Dinerstein 1998) and two biodiversity hotspots (Myers 2003). Despite this importance, the region's fauna remains poorly explored (Ayé et al. 2012). Increased research interest in Central Asia in recent years has resulted in a significant increase in information regarding some taxonomic groups, notably birds (Ayé et al. 2012; Wassink 2015), although little contemporary field-based work has examined the diversity and distributions of other taxa, including reptiles and amphibians (herpetofauna).

Recent information regarding regional-scale distributions and habitat associations of Central Asian amphibian and reptile communities is scarce, with the limited available data focusing on particular countries and habitats. Large-scale herpetofaunal distribution studies have been completed for Turkmenistan (Schkammakov et al. 1993; Tuniyev et al. 1999) and parts of Kazakhstan (Lambert 2002). Trans-national biogeographical patterns for lizard communities in the region's mountains have also been examined (Bobrov 2005). Detailed descriptions, however, remain largely lacking for entire habitat types and countries within Central Asia. Very little community-level information exists on the semi-desert steppe habitats that predominate in non-montane areas of southern Central Asia, and recent outputs from Uzbekistan—the most populous country in the region—are restricted to a small number of species-specific ecology

Correspondence. *tom_martin_2010@yahoo.co.uk

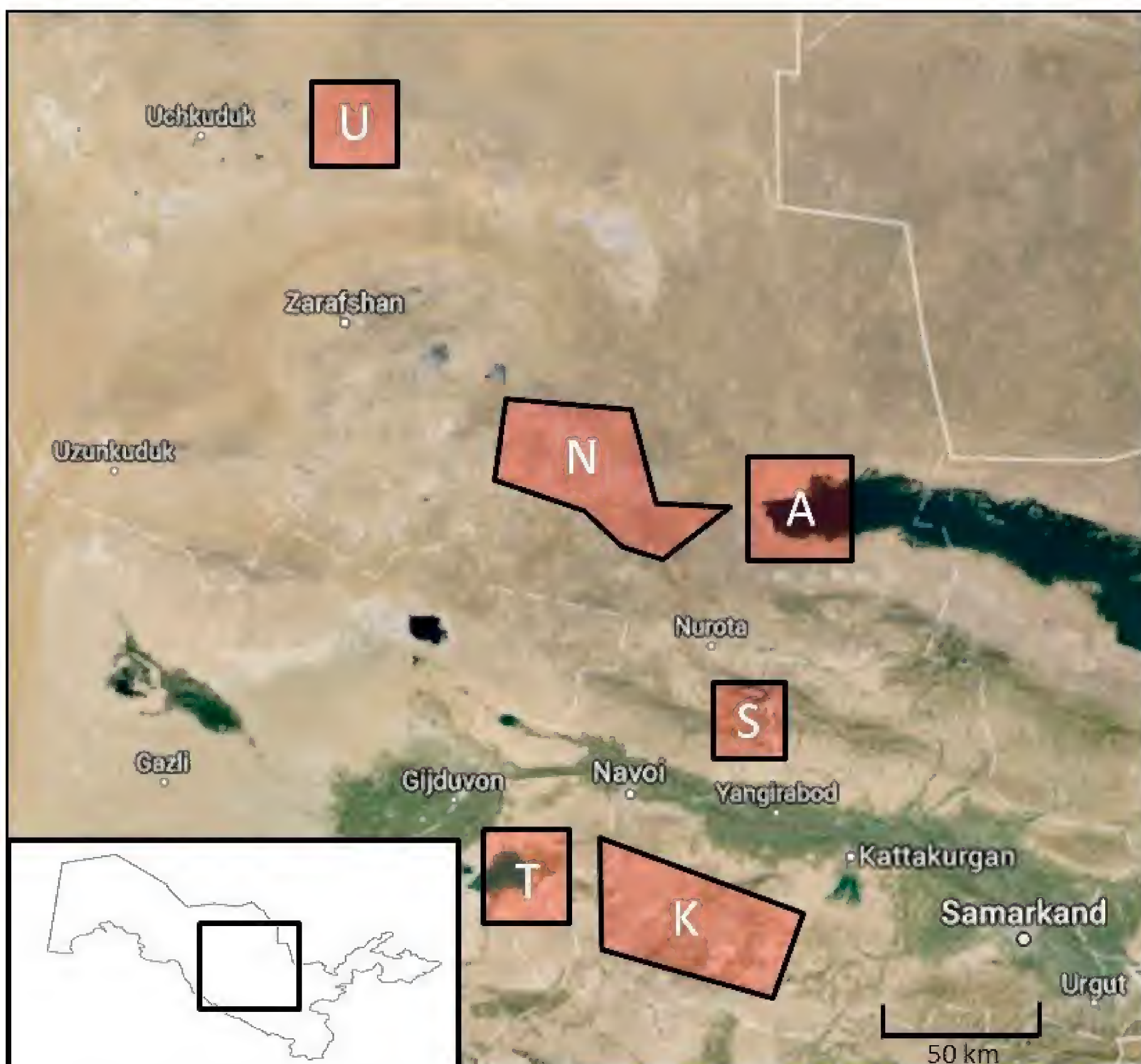


Fig. 1. Map of our study areas within central Uzbekistan. Inset shows the study area within the entirety of Uzbekistan. Notations represent the following locations: K = Karnabchul steppe, N = northern steppe, S = Sarmysh nature park, T = Lake Tudakul, A = Lake Aydarkul, U = Uchkuduk study area.

papers (Lagarde et al. 2002, 2003; Ikramov and Azimov 2004; Clemann et al. 2008). Prior to these, the only existing herpetology resources from Uzbekistan are a number of regional-scale Russian-language texts dating back to the Soviet era, which remain largely inaccessible to the international scientific community (e.g., Bannikov 1971; Rustamov 1981; Rustamov and Shcherbak 1986).

As well as a lack of community-level research, knowledge relating to the statuses of individual species in Uzbekistan is also restricted to a limited range of resources. These include IUCN (2016) species distribution maps (which are lacking for the majority of Central Asian species), coarse-grained spatial range maps provided by The Reptile Database (2016), and a Soviet-era Russian-language text (Bannikov 1971), and regional-scale atlas maps provided in guidebooks to the Western Palearctic as a whole (Sindaco and Jeremcenko 2008;

Sindaco et al. 2013). This general lack of zoological knowledge appears to have had an impact on regional conservation strategies, with steppe and semi-deserts in Central Asia having been noted as being poorly-represented in existing protected area networks (Chemonics International 2001).

In this study we attempt to address this knowledge-gap by providing an annotated checklist of the herpetofauna community of central Uzbekistan, based on opportunistic records made while conducting surveys of the Asian Houbara Bustard (*Chlamydotis macqueenii*). These records represent the first recent data regarding herpetofaunal community composition in this part of Uzbekistan and from the Central Asian semi-desert steppe habitats where survey work was focused. We also provide records from a number of other habitats occurring in the region, notably sand dunes, low mountains, and wetlands.

Materials and Methods

Study site: Fieldwork was concentrated principally in semi-desert grassland habitats (Plate 1) which predominate in central Uzbekistan (Ayé et al. 2012; World Wildlife Fund 2013). This habitat is invariably referred to locally as “steppe.” While true steppe is a less arid ecosystem found in higher latitudes, for ease of reference we henceforth use this term for the semi-desert habitats in our study area. The principal purpose of the authors’ work in these habitats was to monitor populations of Asian Houbara Bustard (*Chlamydotis macqueenii*). Bustard surveys were concentrated in two large expanses of steppe; the Karnabchul steppe region (BirdLife International 2016a) located in southern Navoi province and far western Samarkand province, and a large expanse of steppe located to the north of Navoi city (Fig. 1). While completing this survey work we opportunistically recorded herpetofauna wherever possible (see below). When not committed to completing bustard surveys in the steppe, we surveyed a number of other habitats, most notably the extensive sand dune fields which occur sporadically within our two main steppe study areas, the low mountains of the Sarmysh nature park (BirdLife International 2016c) and the wetland habitats found along the western shore of Lake Aydarkul (BirdLife International 2016b) and eastern shore of Lake Tudakul (BirdLife International 2016d) (Fig. 1). A single five-day visit was also made from 29 April to 03 May 2013 to a large area of steppe and dunes in northern Navoi province, near the city of Uchkuduk, on the fringes of the Kyzylkum desert (Fig. 1).

Altitude throughout this spatially extensive study area ranges from 230 m along the shore of Lake Tudakul to 1,993 m at the highest peak of the mountainous Sarmysh area. Most of the area consists of slightly undulating steppe, however, where elevation typically varies between 300–380 m. The area possesses a continental climate characterized by hot, dry summers, and cold winters with frequent thaws (Glazirin et al. 1999). Mean temperatures vary from 33 °C in July to 1.9 °C in January. Average annual rainfall is approximately 126 mm, with an average of 32 mm falling in February (the wettest month) and <1 mm falling in July (the driest month) (Emirates Centre for the Conservation of Houbara 2013, unpublished data). The area possesses a highly complex geology—the result of its location on a tectonic collision zone (Hendrix and Davis 2001).

Vegetation in steppe habitats is dominated by hardy shrubs of the *Artemisia* genus, interspersed with other shrub assemblages, while sand dune habitats are dominated by a variety of psammophytic plant species (Makhmudovich 2006). Mountainous areas within the Sarmysh Nature Park area possess an Irano-Turanian vegetation assemblage characterized by small, hardy shrubs and trees, notably those of the genera *Prunus* and *Pistacia* (Ayé et al. 2012).

Fieldwork: Herpetofaunal records were made by the authors over the course of three fieldwork seasons spanning the spring (and in one case the early summer) months of 2011, 2012, and 2013. Fieldwork dates ran from 13 March to 27 May in 2011, 04 March to 31 May in 2012, and 15 March to 25 June in 2013. These spring and early summer months represent the optimal time for completing herpetofauna surveys in Central Asia given that most species hibernate during the cold winter months, and that some species return to hibernation prior to the hottest summer months and do not resume activity until the following spring (Lagarde et al. 2003). All records were collected opportunistically, rather than via formalized survey work. These opportunistic records were made in a number of ways. During formal Bustard survey hours within steppe habitats, records were kept of all herpetofaunal species observed while driving between established survey sites during the day, or encountered on foot at these survey sites. Records within steppe habitats were also made driving along roads at night, and from casual exploration during the middle of the day when conditions were not suitable for formal survey work. Exploration was also conducted in sand dune habitats, the low mountains of Sarmysh Nature Park, and the shores of Lake Aydarkul and Tudakul outside of formal survey work. This exploration involved extensive driving and walking on foot in these habitats, both in the day and at night, and noting any records made, as well as targeted searching in microhabitats likely to support specialized herpetofaunal species, such as dune crests, rocky gullies, and well-vegetated river banks. While this opportunistic record making did not follow a systematic survey methodology, herpetofauna species were still actively searched for by the authors, except when formal bustard survey work was being conducted. We estimated the approximate person-hours of survey effort represented by our opportunistic records by calculating the number of days each surveyor spent in the field multiplied by eight (the average number of hours per day each surveyor spent in the field, excluding hours spent conducted formalized bustard counts).

Data analysis: After the completion of survey work we carefully identified all species detected by our survey effort, using all existing field guides and distribution atlases encompassing Central Asia (Bannikov 1971; Sindaco and Jeremcenko 2008; Sindaco et al. 2013). All species identifications were then independently verified by JS. We also sought additional species verifications from Dr. Tatjana Dujsebayaeva at the Kazakhstan Institute of Zoology for all records of *Eryx* Sand boas—a group that can be particularly difficult to separate in the field. We then compiled an inventory of all identified species following the taxonomy provided by Frost (2014) for amphibians and the Reptile Database (2016) for reptiles. We recorded the conservation status of each species in our inventory following the most recent IUCN Red List database (IUCN 2016). We also noted whether each spe-

cies was endemic to Central Asia as defined by species descriptions given by the IUCN (2016).

Categorical abundance estimates for each species were then assigned based on frequency of records. The following categories were used: abundant (typically recorded multiple times every day in suitable habitat); common (typically recorded around once per day); fairly common (typically recorded once per week); uncommon (recorded less than five times per season on average) and rare (known from less than five records overall). A data deficient (DD) category was used for species exclusively found in the Sarmysh mountains which were too briefly explored to provide meaningful abundance estimates.

Finally, we attempted to determine whether our records for each species in our inventory represented an extension to their known spatial range. We assessed two different magnitudes of range extensions: national range extensions for species which had not been previously reported from Uzbekistan, and regional range extensions for species previously reported as occurring in Uzbekistan, but not within our study area, by existing distribution maps. Potential range extensions were assessed by comparing each species in our inventory with records reported in herpetological papers from the region (Lagarde et al. 2002, 2003; Ikramov and Azimov 2004; Clemann et al. 2008), biological summaries of sites of special scientific interest (BirdLife International 2016a,b,c,d), existing distribution maps provided by the IUCN (2016), the Reptile Database (2016), and the two volumes of the only recent species distribution atlas covering Central Asia (Sindaco and Jeremcenko 2008; Sindaco et al. 2013). We also consulted distribution maps provided in Bannikov (1971), although this fairly inaccessible Russian-language text does not provide range maps for all species in the region.

Results

Opportunistic survey effort in our study area constituted approximately 8,680 person-hours. A total of 28 species were detected by this survey effort—two amphibian species and 26 reptile species. All these species are native to the study area, with no current records of introduced or invasive species in the region. Only a single species (*Testudo horsfieldii*) is considered Globally Threatened or Near-Threatened by the IUCN (2016). However, only five species (17.9%) are assigned a definitive threat category—all other species in our inventory remain unasessed or are considered data deficient. No species in our inventory is restricted exclusively to Central Asia, although several, such as the Turkestan Agama (*Paralaudakia lehmanni*), are almost entirely confined to the region and therefore considered “near-endemic.” All species we detected were previously known to occur in Uzbekistan (IUCN 2016; Reptile Database 2016), thus we report no national range extensions. However, records for five species (*Eremias scripta*, *Eryx miliaris*, *Hemorrhois ravergieri*, *Natrix tessellata*, and *Echis carinatus*) represent regional range extensions within central Uzbekistan. A full summary of species detected in our study area is provided in Table 1, with the descriptions below providing more detailed information for each species in our inventory.

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AMPHIBIANS

Green Toad: *Bufotes viridis* (Laurenti 1768) (Bufonidae) (Plate 5)

A widespread species found from western Europe to Kazakhstan. The taxonomy of species appears to be unclear, with some sources splitting Central Asian populations as *B. variabilis*, and others separating the populations of the southern and eastern Central Asian steppes still further as *B. pewzowi* (Ficetola and Stöck 2016), although we retain the Frost (2013) nomenclature. A nocturnal species, it is locally abundant in our study area, and is typically found around permanent and ephemeral water sources throughout the area's steppe habitats. However, individuals were also occasionally encountered in the open steppe at least one km from any known water sources. The species has been previously reported as occurring in the study area (Bannikov 1971; BirdLife International 2016c).

Marsh Frog: *Pelophylax ridibundus* (Pallas 1771) (Ranidae) (Plate 6)

A widely distributed species found throughout much of Europe and western Asia. It is abundant in unpolluted, non-saline water sources, particularly in agricultural fields and drainage ditches located on the peripheries of steppe habitats and the two large lake ecosystems, and small mountain streams in the Sarmysh region. Unlike *B. viridis*, this species was never observed far away from water. *Pelophylax ridibundus* is previously known to occur within the study area (Bannikov 1971; BirdLife International 2016c).

REPTILES

Russian Tortoise: *Testudo horsfieldii* (Gray 1844) (Testudinidae) (Plate 7)

The only Chelonian species found in our study area, *T. horsfieldii* is restricted to Central Asia and Iran, and is listed as Vulnerable by the IUCN (2016). The species is abundant in the steppe habitats of our study area, with multiple individuals typically being seen every day in this habitat from between early March when they emerge from hibernation to mid-June, when their hibernation resumes (Lagarde et al. 2003). It was by a large margin the most frequently encountered herpetofaunal species during the course of our fieldwork. The species has been previously mapped as occurring in central Uzbekistan (Bannikov 1971; Sindaco and Jeremcenko 2008; BirdLife International 2016a–d) although the atlas map in Sindaco and Jeremcenko (2008) does not note its pres-

Table 1. Summary of amphibian and reptile species recorded in our central Uzbekistan study area between 2011 and 2013. New range extensions are marked in bold. Species marked † are assessed as Threatened or Near Threatened by the IUCN (2016). Abundance estimates are denoted as follows: A = abundant; C = common; Fc = fairly common; U = uncommon; R = rare; DD = data deficient. Broad locales in the “locations” column are denoted as follows: A = Lake Aydarkul; K = Karnabchul steppe; N = northern steppe area; S = Sarmysh nature park; T = Lake Tudakul; U = Uchkuduk area. All taxonomy follows Frost (2014) for amphibians and the Reptile Database (2016) for reptiles.

Class	Order	Family	Common name	Scientific name	Abundance	Locations
Amphibia	Anura	Bufoidae	Green Toad	<i>Bufo viridis</i>	A	K, N
		Ranidae	Marsh Frog	<i>Pelophylax ridibundus</i>	A	K, S
Reptilia	Testudines	Testudinidae	†Russian Tortoise	<i>Testudo horsfieldii</i>	A	K, N, U
	Squamata (Sauria)	Agamidae	Turkestan Agama	<i>Paralaudakia lehmanni</i>	Fc	K, S
			Brilliant Ground Agama	<i>Trapelus agilis</i>	A	K, N
			Sunwatcher Toadhead Agama	<i>Phrynocephalus helioscopus</i>	Fc	K, N
			Secret Toadhead Agama	<i>Phrynocephalus mystaceus</i>	U	N, U
			Lichtenstein’s Toadhead Agama	<i>Phrynocephalus interscapularis</i>	Fc	N
		Geckkonidae	Common Wonder Gecko	<i>Teratoscincus scincus</i>	Fc	N
			Russian Bent-toed Gecko	<i>Tenuidactylus fedtschenkoi</i>	Fc	K
			Caspian Bent-toed Gecko	<i>Tenuidactylus caspius</i>	Fc	K, N
		Lacertidae	Striped Racerunner	<i>Eremias lineolata</i>	U	N, U
			Rapid Fringe-toed Lizard	<i>Eremias velox</i>	A	K, N
			Reticulate Racerunner	<i>Eremias grammica</i>	U	N, U
			Sand Racerunner	<i>Eremias scripta</i>	R	N, U
		Scincidae	Berber Skink	<i>Eumeces schneideri</i>	DD	S
		Anguidae	European Glass Lizard	<i>Pseudopus apodus</i>	DD	S
		Varanidae	Desert Monitor	<i>Varanus griseus</i>	C	K, N, U
	Squamata (Serpentes)	Boidae	Tartar Sand Boa	<i>Eryx tataricus</i>	U	K, N
			Dwarf Sand Boa	<i>Eryx miliaris</i>	U	K, N
		Colubridae	Spotted Desert Racer	<i>Platyceph karelini</i>	U	K, N
			Wadi Racer	<i>Platyceph rhodorachis</i>	R	K
			Diadem Snake	<i>Spalerosophis diadema</i>	U	K
			Spotted Whipsnake	<i>Hemorrhoids ravigieri</i>	R	N
		Lamprophiidae	Steppe Ribbon Racer	<i>Psammophis lineolatus</i>	Fc	K, N
		Natricidae	Dice Snake	<i>Natrix tessellata</i>	U	A, T
		Elapidae	Central Asian Cobra	<i>Naja oxiana</i>	U	K, S
		Viperidae	Saw-scaled Viper	<i>Echis carinatus</i>	C	K, N

ence in the Uchkuduk area, where our records indicate the species to occur.

Turkestan Agama: *Paralaudakia lehmanni* (Nikolsky 1896) (Agamidae) (Plate 8)

Found only in southern Central Asia and Pakistan. *Paralaudakia lehmanni* is a fairly common species in suitable habitats within our study area, being regularly sighted on boulders and crevasses in rock faces within low mountain habitats on the periphery of the Karnabchul steppe and in the Sarmysh Nature Park. The species has been previously mapped as occurring in the study area (Sindaco and Jeremcenko 2008).

Brilliant Ground Agama: *Trapelus agilis* (Olivier 1807) (Agamidae) (Plate 9)

A widespread species found in the Caucasus, much of Central Asia, Iran, and the Indian subcontinent. *Trapelus agilis* is abundant in our study area, being typically observed multiple times per day in open steppe habitats and in the low mountains on the edge of the Karnabchul area, once the species has emerged from hibernation in mid-late March. It is frequently observed basking on top of Artemisia shrubs. *Trapelus agilis* has been previously mapped as occurring in our study area (Sindaco and Jeremcenko 2008; BirdLife International 2016a–d).

Sunwatcher Toadhead Agama: *Phrynocephalus helioscopus* (Pallas 1771) (Agamidae) (Plate 10)

This species is found in southern European Russia and much of Central Asia. It is fairly common in the open steppe habitats of our study area, and has occasionally



Plate 1. Semi-arid “steppe” habitats (*Photograph – CL*).



Plate 2. Vegetated sand-dune habitats (*Photograph – CL*).



Plate 3. Low rocky mountains (*Photograph – JD*).



Plate 4. Wetland habitats as Lake Aydarkul (*Photograph – RE*).



Plate 5. *Bufo viridis* (*Photograph – TM*).



Plate 6. *Pelophylax ridibundus* (*Photograph – TM*).



Plate 7. *Testudo horsfieldi* (*Photograph – TM*).



Plate 8. *Paralaudakia lehmanni* (*Photograph – TM*).

been observed in sand dunes. It is previously recorded as occurring throughout central Uzbekistan (Bannikov 1971; Sindaco and Jeremcenko 2008; IUCN 2016; BirdLife International 2016a,c).

Secret Toadhead Agama: *Phrynocephalus mystaceus* (Pallas 1776) (Agamidae) (Plate 11)

This distinctive, large-bodied agama possesses a wide but patchy distribution across southern European Russia, much of Central Asia, and eastern Iran. A strict sand dune species, it is uncommon in our study area, typically being observed only once or twice per research season in very specific areas, such as the dune fields within the northern steppe area and in the vicinity of Uchkuduk where we observed both single individuals and apparent pairs. The species has been previously mapped as occurring throughout our study area (Sindaco and Jeremcenko 2008).

Lichtenstein's Toadhead Agama: *Phrynocephalus interscapularis* (Lichtenstein 1856) (Agamidae) (Plate 12)

A small agamid found in all the Central Asian Republics and the northern border areas of Iran. *Phrynocephalus interscapularis* is fairly common in our study area. As with *P. mystaceus* it is restricted to sand dunes, although the two species display strong niche separation within this habitat. Clemann et al. (2008) describe how *P. mystaceus* occupies dune crest microhabitats, while *P. interscapularis* occupies mid-dune microhabitats and swales between dunes—a pattern our observations corroborate. The species has been previously mapped as occurring throughout our study area (Sindaco and Jeremcenko 2008).

Common Wonder Gecko: *Teratoscincus scincus* (Schlegel 1858) (Gekkonidae) (Plate 13)

A widespread species found throughout Central Asia, Iran, Pakistan, and parts of the Arabian Peninsula. *Teratoscincus scincus* is a strictly nocturnal species which appears to be fairly common within our study area (although this abundance estimate could be a product of our sampling effort—see Discussion). We only detected *T. scincus* in the northern steppe habitats of our study area, where it was encountered regularly on roads while driving at night, and occasionally on foot when walking in the open steppe after dusk. The species is indicated to occur throughout our study area by Bannikov (1971), although our records represent a slight range extension to the atlas maps produced by Sindaco and Jeremcenko (2008).

Russian Bent-toed Gecko: *Tenuidactylus fedtschenkoi* (Strauch 1887) (Gekkonidae) (Plate 14)

Restricted to Central Asia and northern border areas of Pakistan and Iran. *Tenuidactylus fedtschenkoi* is fairly common in rocky mountain habitats on the peripheries of

our two main steppe study areas, as well as on isolated rocky outcrops within the steppe. The species was almost always observed within a short distance of holes and crevasses in the rock face, where they retreated when disturbed. *Tenuidactylus fedtschenkoi* has been previously mapped as occurring in our study area (Bannikov 1971; Sindaco and Jeremcenko 2008; BirdLife International 2016a,c).

Caspian Bent-toed Gecko: *Tenuidactylus caspius* (Eichwald 1831) (Gekkonidae) (Plate 15)

A widespread species found throughout southern Central Asia and around the basin of the Caspian Sea. *Tenuidactylus caspius* is common in our study area, being found in a variety of habitats including cliff faces, isolated rocky outcrops in the steppe, abandoned ruins, and within inhabited buildings. It is mapped as occurring throughout our study area by the IUCN (2016), although our records represent modest extensions to the distribution maps provided by Bannikov (1971) and Sindaco and Jeremcenko (2008).

Striped Racerunner: *Eremias lineolata* (Nikolsky 1897) (Lacertidae) (Plate 16)

This species is found throughout southern Central Asia and in north-eastern Iran. It is uncommon in our study area, being found only in extensive areas of vegetated sand dunes, often alongside populations of *E. scripta* and *E. grammica*. It has been previously mapped as occurring throughout our study area (Sindaco and Jeremcenko 2008; BirdLife International 2016d).

Rapid Fringe-toed Lizard: *Eremias velox* (Pallas 1771) (Lacertidae) (Plate 17)

A widespread species found throughout Central Asia, southern European Russia, and Iran. *Eremias velox* is abundant in our study area and was by far the most frequently observed *Eremias* species, occurring at high densities throughout the region's open steppe habitats. The species has been previously mapped as occurring throughout our study area (Sindaco and Jeremcenko 2008).

Reticulate Racerunner: *Eremias grammica* (Lichtenstein 1823) (Lacertidae) (Plate 18)

Distributed across Central Asia, western China, and north-eastern Iran, *E. grammica* is an uncommon species in our study area. It was only observed in vegetated sand dune habitats similar to those inhabited by *E. lineolata* and *E. scripta*. It has been previously reported as occurring throughout the study area (Bannikov 1971; Sindaco and Jeremcenko 2008).

Sand Racerunner: *Eremias scripta* (Strauch 1867) (Lacertidae) (Plate 19) *Regional range extension

A widely, although patchily, distributed species found throughout Central Asia, western China, Iran, and Paki-



Plate 9. *Trapelus agilis* (Photograph – TM).



Plate 10. *Phrynocephalus helioscopus* (Photograph – VNM).



Plate 11. *Phrynocephalus mystaceus* (Photograph – RE).



Plate 12. *Phrynocephalus interscapularis* (Photograph – VNM).



Plate 13. *Teratoscincus scincus* (Photograph – TM).



Plate 14. *Tenuidactylus fedtschenkoi* (Photograph – TM).

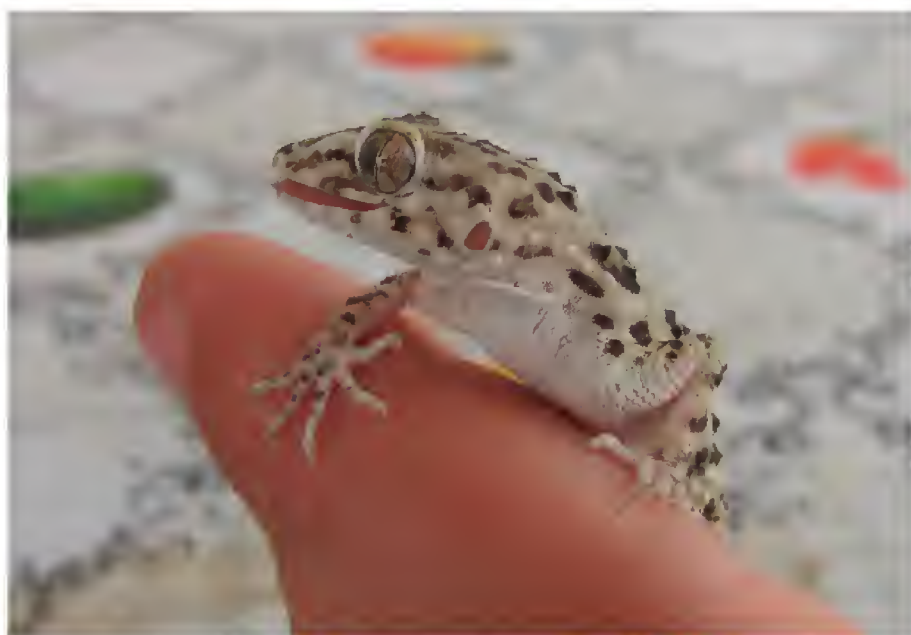


Plate 15. *Tenuidactylus caspius* (Photograph – TM).



Plate 16. *Eremias lineolata* (Photograph – MG).

stan. It is rare within our study area, having been recorded a total of four times. It appears to inhabit similar habitats to *E. lineolata* and *E. grammica*, being found locally within well-vegetated sand dune fields. While *E. scripta* has been noted as occurring at Lake Tudakul (BirdLife

International 2016d), our records of this species in the northern steppe and Uchkuduk areas represent regional range extensions to its known distribution, with none of our consulted sources noting its occurrence in these areas of central Uzbekistan.

Berber Skink: *Eumeces schneideri* (Daudin 1802) (Scincidae) (Plate 20)

A widespread species found from North Africa to the Indian sub-continent. Within our study area, the species is known from a single individual found on rocky scree in a canyon within the mountains of the Sarmysh Nature Park on 26 May 2012. As we only spent a few days prospecting for herpetofauna in the Sarmysh area, it is hard to ascertain whether this species is genuinely rare here, or whether it is more common than our limited survey effort in appropriate habitats suggests. At the very least, it appears to be very localized and habitat-specific within central Uzbekistan. We never encountered the species in the steppe or in mountainous ecosystems immediately adjacent to the steppe (unlike other rocky habitat specialists such as *Paralaudakia lehmanni* and *Temnidactylus fedtschenkoi*). The species has been previously mapped as occurring in our study area (Sindaco and Jeremcenko 2008; BirdLife International 2016c).

European Glass Lizard: *Pseudopus apodus* (Pallas 1775) (Anguidae) (Plate 21)

A widespread species found from eastern Europe to eastern Kazakhstan. Within our study area it is known only from three records of live individuals in tall grass meadows in close proximity to streams within the Sarmysh Nature Park, and four dead individuals seen in villages on the outskirts of Sarmysh (two having been hit by vehicles, one being carried in a plastic bag by a local, and one found washed up on the bank of a small river). As with *Eumeces schneideri*, the species appears to be very localized within our study area, but our low survey effort within the Sarmysh mountains makes it difficult to accurately estimate its relative abundance within its limited range. It has been previously mapped as occurring in our study area (Bannikov 1971; Sindaco and Jeremcenko 2008; BirdLife International 2016c).

Desert Monitor: *Varanus griseus* (Daudin 1803) (Varanidae) (Plate 22)

This large-bodied monitor lizard is widely distributed in arid habitats from north Africa to the Indian Subcontinent. It is a common species in our study area, typically being observed at least once per day in open steppe habitat and sand dunes from mid-April onwards, when the species emerges from hibernation. It has been previously recorded as occurring throughout our study area by Bannikov (1971) although Sindaco and Jeremcenko (2008) do not map its occurrence in the Uchkuduk area, where we observed the species several times.

Tartar Sand Boa: *Eryx tataricus* (Lichtenstein 1823) (Boidae) (Plate 23)

A widespread Asian species found from Iran through southern Central Asia to western China and Mongolia. It is an uncommon inhabitant of open steppes and sand dunes in our study area. Its appearance seems to

be highly variable in central Uzbekistan. The species has been previously mapped as occurring throughout central Uzbekistan (Sindaco et al. 2011).

Dwarf Sand Boa: *Eryx miliaris* (Pallas 1773) (Boidae) (Plate 24) *Regional range extension

A less widely-distributed species than *E. tataricus*. *Eryx miliaris* is largely confined to Central Asia, with its range extending slightly into Iran and southern European Russia. An uncommon species in our study area, it was detected about as frequently, and in similar habitats to, *E. tataricus*. Our records of this species constitute a regional range extension. BirdLife International (2016d) noted its presence at Lake Tudakul, but none of our consulted sources indicate the species to occur in Karnabchul or the northern steppe areas where we have detected it.

Spotted Desert Racer: *Platyceps karelini* (Brandt 1838) (Colubridae) (Plate 25)

This species is found in all the Central Asian Republics, Iran, and Pakistan. It is uncommon in our study area, being occasionally recorded in the open steppe and sand dune habitats of Karnabchul and the northern steppe areas. It has been previously mapped as occurring in our study area (Bannikov 1971; Sindaco et al. 2011; BirdLife International 2016d).

Wadi Racer: *Platyceps rhodorachis* (Jan 1865) (Colubridae) (Plate 26)

A widely but disjunctively distributed species, found in east Africa, Arabia, Iran, Central Asia, and the northern Indian sub-continent. It appears to be rare in our study area, being known from a single predated and partially consumed individual (see Plate 26) found on the northern border of the Karnabchul steppe, close to a range of rocky foothills, on 17 May 2012. The species has been previously mapped as occurring in our study area (Bannikov 1971; Sindaco et al. 2011).

Diadem Snake: *Spalerosophis diadema* (Schlegel 1837) (Colubridae) (Plate 27)

A widely distributed species occurring from west and north Africa throughout the Middle East to Central Asia. This fairly large snake species is uncommon in our study area, typically being observed two or three times per season in open steppe and in close proximity to inhabited areas. The species is noted as occurring throughout the study area by Bannikov (1971), although Sindaco et al. (2011) do not map its occurrence in central Uzbekistan.

Spotted Whipsnake: *Hemorrhois ravergieri* (Mènètries 1832) (Colubridae) (Plate 28) *Regional range extension

Distributed in Turkey, the Caucasus, Iran, Central Asia, and western China. It is a rare species in our study area, known from a single record of an individual observed in open steppe habitat on 11 May 2011 in



Plate 17. *Eremias velox* (Photograph – TM).



Plate 18. *Eremias grammica* (Photograph – RE).



Plate 19. *Eremias scripta* (Photograph – MG).



Plate 20. *Eumeces schneideri* (Photograph – TM).



Plate 21. *Pseudopus apodus* (Photograph – RE).



Plate 22. *Varanus griseus* (Photograph – RE).



Plate 23. *Eryx tataricus* (Photograph – TM).



Plate 24. *Eryx miliaris* (Photograph – MG).

our northern steppe study area. This record represents a regional range extension for *H. ravergeri*. The species is not mapped as occurring in central Uzbekistan by Bannikov (1971) and Sindaco et al. (2011), and while its presence has been noted in the Lake Tudakul area (BirdLife 2016d), it does not appear to have been previously reported as occurring further north in Uzbekistan.

Steppe Ribbon Racer: *Psammophis lineolatus* (Brandt 1838) (Lamprophiidae) (Plate 29)

Found in all the Central Asian Republics, western China, and Iran. *Psammophis lineolatus* is fairly common in the steppe and sand dune habitats of our study area, being observed on average about once per week. The species was, overall, the most frequently observed snake species during the course of our fieldwork. The species has been previously mapped as occurring throughout our study area (Bannikov 1971; Sindaco et al. 2011).

Dice Snake: *Natrix tessellata* (Laurenti 1768) (Natricidae) (Plate 30) *Regional range extension

A widespread species ranging from central Europe to Egypt, the Middle East, and western China. It was uncommon within our study area, typically being observed a few times per season in freshwater habitats along the shores of Lake Aydarkul and Lake Tudakul, as well as in irrigation canals in cultivated land in the vicinity of Tudakul. Our records for this species represent a regional range extension to its known distribution. Bannikov (1971) and Sindaco et al. (2011) do not map its presence anywhere in our study area. BirdLife International (2016d) notes its presence at Lake Tudakul, but our records from the western shore of Lake Aydarkul appear to be entirely new.

Central Asian Cobra: *Naja oxiana* (Eichwald 1831) (Elapidae) (Plate 31)

Restricted to southern Central Asia and borders areas of Iran and the Indian sub-continent. *Naja oxiana* is an uncommon species in our study area. It was typically observed about once per season in steppe habitats close to low foothills in the Karnabchul area (including one dead individual found on a road measuring 156 cm), and in the low mountains of Sarmysh. The species has been previously noted as occurring throughout the study area (Bannikov 1971; Sindaco et al. 2011; BirdLife International 2016a,c).

Saw-scaled Viper: *Echis carinatus* (Schneider 1801) (Viperidae) (Plate 32) *Regional range extension

A widespread species found in the Middle East, Iran, Central Asia, and the Indian sub-continent. This highly venomous viper is common in suitable habitats within our study area, being observed almost daily within a few specific areas of human habitation on the edge of the Karnabchul area, and occasionally in the open habitats of Karnabchul and the northern steppe areas. Our records represent a regional range extension for *E. carinatus*.

Bannikov (1971) only maps the species as occurring along Uzbekistan's western border with Turkmenistan, while Sindaco et al. (2011) note its occurrence only in the south-west and extreme north of the country.

Discussion

The results presented in this paper represent by far the most extensive recent account of Uzbekistan's herpetofauna, resulting from nearly 8,700 person-hours of observational sampling. Our results are not only based on significant survey effort, but are also derived from extensive surveys encompassing a broad and representative range of Central Uzbekistan's habitats, in contrast to other recent papers from the country (i.e., BirdLife International 2016a–d; Lagarde et al. 2002, 2003; Ikramov and Azimov 2004; Clemann et al. 2008). Thus, this study provides a much-needed update to the understanding of the diversity and distributions of the region's understudied herpetofauna, especially with regards to the five reported regional range extensions. The results of this study allow for some appreciation of the relative diversity of the central Uzbekistan herpetofauna compared to that found in surrounding countries. While differences in sampling effort and scope need to be considered, the 28 species detected in our study area do not appear to represent as diverse a species assemblage as steppe/foothill mosaic landscapes found further south in Turkmenistan. In Turkmenistan, 49 species have been recorded from a broadly comparable site (Tuniyev et al. 1999) but do seem to support higher overall richness compared to a steppe/foothills site in southern Kazakhstan, further to the north (Lambert 2002), where just 17 species were recorded. This tentatively suggests that a latitudinal diversity gradient exists across the Turan Plain—the biogeographical zone encompassing most non-mountainous areas of Central Asia (Djamali et al. 2010). Determining the precise delimitations of such a gradient may represent an interesting avenue for future regional research. The results of this study also highlight the extent to which the conservation status of the Central Asian herpetofauna remains heavily neglected. For example, our review of IUCN (2016) classifications revealed that only 18% of species in the area possess definitive threat evaluations. An improved understanding of Central Asia's herpetofauna—and biodiversity in general—is therefore crucial in order to both better understand the consequences of, and to mitigate, the heavy environmental pressures facing the region. Regionally, key threats to the Central Asian herpetofauna include climate change and habitat degradation due to overgrazing and other unsustainable land uses (Christensen et al. 2004; Lioubimtseva et al. 2005), as well as unsustainable collection for the international pet trade (Kuzmin 1994; Cheung and Dudgeon 2006; Robinson et al. 2015). With regards to our central Uzbekistan study sites specifically, habitats are also threatened by extensive mining operations seeking to



Plate 25. *Platyceps karelini* (Photograph – MG).



Plate 26. *Platyceps rhodorachis* (Photograph – CL).



Plate 27. *Spalerosophis diadema* (Photograph – MG).



Plate 28. *Hemorrhois ravergieri* (Photograph – RE).



Plate 29. *Psammophis lineolatus* (Photograph – MG).



Plate 30. *Natrix tessellata* (Photograph – TM).



Plate 31. *Naja oxiana* (Photograph – TM).



Plate 32. *Echis carinatus* (Photograph – TM).

exploit the abundant mineral resources (including gold, uranium, and natural gas) found throughout much of the area (F. Andrianova, pers. comm.). The relative impacts of these threats are expected to differ between habitats. Species well adapted to human-modified landscapes (notably *T. caspius* and *E. carinatus*) or rocky hills (such as *T. fedtschenkoi*, *P. lehmanni*, and *E. schneideri*) are in likelihood less vulnerable to habitat loss and degradation than species occurring exclusively in the steppe, where grazing and mining activities are concentrated.

While the results of this study represent a valuable contribution to regional herpetological knowledge, these records exhibit a strong bias towards the steppe ecosystems forming the focus of our simultaneous Bustard survey work. While considerable observational effort was directed towards central Uzbekistan's other major habitats (with the exception of the mountains of Sarmysh—see Materials and Methods), they were not explored to the same extent as the steppe. This could mean that some of the relative abundance values assigned for non-steppe species in Table 1 are influenced by reduced survey effort in dune, wetland, and mountain habitats, and thus underestimate true relative abundances. As the bulk of our observations were made during daylight hours, this could also be true for strictly nocturnal species, such as *Teratoscincus scincus* or *Echis carinatus*. Concentrating survey effort in the spring may also have led to underestimating the relative abundance of any species possessing peak activity periods in the summer months.

Reduced survey efforts in non-steppe habitats may have led to some species in these areas being unrecorded due to a simple lack of detection. For example, the Blunt-nosed Viper (*Macrovipera lebetina*) was not conclusively observed during our fieldwork, but has been previously recorded as occurring in central Uzbekistan (Bannikov 1971; BirdLife International 2016c). A long (50 cm>), fat-bodied snake carcass observed along a roadside in low hills near the village of Kyzulkuduk in the northern steppe area in May 2011 may have been this species. However, this specimen was not closely examined and no photograph was taken to verify its identity. Other species indicated to potentially occur in the region, but were not detected by our survey effort, include *Eremias arguta*, *Crossobamon eversmanni*, and *Gloydus halys* (Bannikov 1971; Sindaco et al. 2011).

Conclusions

This study provides a valuable overview of the diverse herpetofaunal community of central Uzbekistan. However, further work in the area is required to extend the region's species inventory, and subsequently provide more accurate species abundance estimates, while improving knowledge of species-habitat relationships. Further field surveys are encouraged to focus on the region's non-steppe habitats (especially montane ecosystems), which were under-represented by survey effort in

this study, and to employ more systematic survey methods than were possible here. This study highlights the significant lack of information regarding the conservation status of most species occurring in the steppes and other habitats of central Uzbekistan, and we strongly recommend that IUCN threat status auditors utilize all available resources to address the apparent knowledge gap occurring in this part of Central Asia.

Acknowledgements.—This project was completed under the supervision of the Emirates Centre for the Conservation of the Houbara (ECCH), which is managed by Reneco for Wildlife Preservation (www.reneco.org). We greatly thank H.H. Sheikh Mohammed Bin Rashid Al Maktum, funder of the ECCH, for his support. We are also grateful for the support of Frédéric Lacroix, Mohamed Beljafla, Adeline Cadet, and Yves Hingrat, respectively general manager of, director of ECCH, project manager of ECCH, and head of Reneco's ecology and conservation department. We also thank staff involved in methods design and data collection—particularly ecology coordinators: Eric Le Nuz and Cédric Ferlat, and field workers: Andy Simpkin, Olga Lukshyts, Vladimir Bezmelnitsyn, Alfonso Godino, Yury Bakur, Jesse Gabbard, Edward Mongin, Maksim Tarantovich, and Valerie Dombrovski. Finally, we extend thanks to Dr Tatjana Dujsebayaeva of the Kazakhstan Institute of Zoology for assistance with verifying our species records of *Eryx* Sand Boas, and two anonymous reviewers for their useful and constructive comments.

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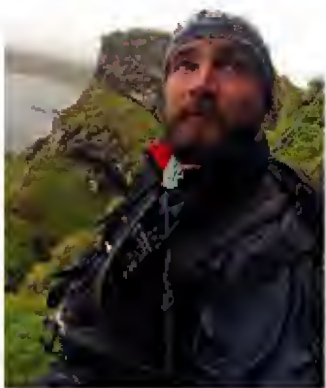
The herpetofauna of central Uzbekistan



Thomas Edward Martin is a conservation biologist based with Reneco for Wildlife Preservation, UAE. He has an interest in the biogeography and ecology of species inhabiting poorly-explored parts of the tropics and sub-tropics, in particular the steppes of Central Asia and the rainforests of Indonesia.



Mathieu Guillemain is a field biologist based with Reneco for Wildlife Preservation, UAE. He has a long-standing interest in the herpetofauna of the arid zones of North Africa and Central Asia, having spent nearly a decade completing fieldwork in these ecosystems. He currently spends a large part of the year as a project manager in the Betpak-Dala steppe, Kazakhstan.



Valentin Nivet-Mazerolles is a conservation biologist with a wide range of interests, having recently completed varied forms of fieldwork in Morocco, Uzbekistan, Kazakhstan, and Crozet Island in the Southern Indian Ocean. He currently works for the French National Reserve service in the Jura region.



Cecile Landsmann is a field biologist based with Reneco for Wildlife Preservation, UAE. She has worked for many years in the arid steppe ecosystems of North Africa and Central Asia, and has developed a strong broad interest in the ecology of these regions. She currently spends much of her year working as a project manager in Central Uzbekistan.



Jerome Dubos is an experienced conservation biologist who first worked as a field technician in the Central Asian steppes in 2009, and has returned to the region most years since. He also has extensive experience conducting field surveys in France, North Africa, and the Middle East. He is currently working on the LIFE+ Petrels project on Reunion Island, working towards the conservation of the two endemic Petrel species found there.



Rémy Eudeline is an enthusiastic herpetologist and a biology teacher, currently based on Mayotte Island in the Indian Ocean. He has developed a strong interest in the herpetofauna of Mayotte, in particular its poorly-studied endemic blind snakes.



James T. Stroud is a Ph.D. candidate at Florida International University. He has a broad range of research interests, often revolving around investigating how ecological processes may explain evolutionary patterns, most commonly using herpetofauna as model species and study systems. He has extensive herpetological fieldwork experience in Europe, the Neotropics, and South-East Asia.